



**EFFECT OF ZINC SULPHIDE NANOPARTICLES ON GERMINATION
OF SEEDS OF *VIGNA RADIATA* AND THEIR SUBSEQUENT
ACCELERATION OF GROWTH IN PRESENCE OF THE
NANOPARTICLES**

Sutapa Ganguly¹, Sukhen Das¹, Sujata G.Dastidar^{2*}

¹Department of Physics, Jadavpur University, Kolkata- 700 032, India.

²Department of Microbiology, Herbicure Healthcare Bio-Herbal Research Foundation,
D.H. Road, Pailan, Kolkata- 700 104, India.

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***Correspondence for
Author**

Sujata G.Dastidar

Department of Microbiology,
Herbicure Healthcare Bio-
Herbal Research Foundation,
D.H. Road, Pailan, Kolkata-
700 104, India

ABSTRACT

The synthesis, characterization and biological application of synthesized nanomaterials have become an important branch of nanotechnology. This study describes the synthesis of highly dispersed zinc sulphide nanoparticles using a simple aqueous chemical method. Such synthesized nanoparticles were tested for their effect on germination of seeds and on acceleration of seedling growth. Scanning electron microscopy (SEM) micrograph analysis of the zinc sulphide nanoparticles (ZNPs) indicated that they were well dispersed and

ranged in size from 10-30 nm. ZNPs were employed to improve germination of seeds and rate of seedling growth of *Vigna radiata*. Three sets of seeds were allowed to germinate on water with two different concentrations (10 to 20 mg/ml) of ZNPs. Higher percentage (70%) of germination was found in treated seeds when compared to the control. The seeds that were in a Petri plate with sterile distilled water only took longer time (1- 2 days) to sprout, whereas all treated seeds sprouted within 6 hr. The maximum height (12.8 cm) was observed in seedlings treated with 20 mg/ml of ZNPs. The possible contribution of ZNPs was to facilitate the penetration of water and nutrients through the seed coat and accelerate the germination of seeds.

KEYWORDS: zinc sulphide, germination of seeds, Nanoparticles, *Vigna radiata*.

INTRODUCTION

Nanotechnology is a versatile field and has found applications in almost all existing fields of science. Application of nanotechnology is now available in various fields of science due to the extensive research being undertaken through out the world. Nanotechnology has the potential to revolutionize agriculture with new tools to enhance the ability of plants to absorb specific required nutrients ^[1]. Nanoparticles are known to have interactions at molecular levels in living cells and nano agriculture involves the employment of nanoparticles in agriculture with the hope and ambition that these particles may have an impact on some beneficial effects in the crops ^[2]. The use of nanoparticles in growth of plants and for the control of plant diseases is a rather recent practice ^[3-4]. Nanoparticles of size below 100 nm fall in the transition zone between individual molecules and the corresponding bulk materials, which generates both positive and negative biological effects in living cells ^[5]. However, interest in research have been increasing on the biological effects of nanoparticles on higher plants. Lu *et al.*,^[6] studied the effect of mixtures of nano SiO₂ and nano TiO₂ on soybean seeds. They found that the mixture of nano particles could enhance nitrate reductase in soybeans increasing its rate of germination and growth; and observed the action of ZnO on growth of *Vigna radiata* and *Cicer arietinum* seedlings using plant agar method ^[7] and peanuts ^[8]. Single walled carbon nanotubes (SWNTs) are known to have the capacity to transverse across both the plant cell wall and cell membrane ^[9]. Gonzales-Melendi *et al.*^[10] reported that the nanoparticles were able to act as smart treatment delivery systems in plants. Compared to plant cell walls and membranes the penetration of nanoparticles into seeds may turn out to be difficult due to thickness of seed coats ^[11]. In spite of this carbon nanotubes could effectively penetrate seed coat and influence the seed germination and plant growth ^[12].

Our earlier studies had shown that ZnS Nanoparticles synthesized by a simple aqueous chemical process possess distinct antimicrobial action ^[13].

Further studies with the same ZnS Nanoparticles proved that such particles could potentiate the antibacterial action of the anticancer agent oxaliplatin ^[14].

Mung bean, also known as mung dal, moong dal, mash bean, munggo or monggo, green gram, golden gram, and green soy, is the seed of *Vigna radiata* which is native to India. The beans are small, ovoid in shape, and green in color. The English word "mung" was derived from the Hindi word mung. In the Southern parts of India in the Tamil language it is known as payiru and in Kannada language the same is called hesaru bele. However, in the

Philippines the same is referred as munggo or monggo. The mung bean is one of many species recently moved from the genus *Phaseolus* to *Vigna* and is still often seen cited as *Phaseolus aureus* or *Phaseolus radiatus* (Old name). The present study describes the effect of synthesized ZNPs on seed germination and formation of early seedlings of *Vigna radiata*.

MATERIALS AND METHODS

Seeds: The seeds of *Vigna radiata* were purchased in sealed packets from the local market.

Chemical compounds: Analar ZnCl₂ and Na₂S were purchased from Merck, Germany, these were allowed to react to produce ZnCl₂ nanoparticles.

Media: liquid media used for the study was sterile distilled water.

Method of preparation of ZnS nanoparticles

Synthesis of ZnCl₂ nanoparticles was carried out by aqueous chemical method using ZnCl₂ and Na₂S as source materials. All the reagents were of analytical grade and used without further purification. The entire process was carried out in distilled water for its inherent advantages of being simple and environment friendly. All steps of the synthesis were performed at 28°C temperature and ambient conditions. In a typical preparation solution of 1M Na₂S was added drop by drop to 1M ZnCl₂ solution which was kept on stirring using a magnetic stirrer at 70 °C for 2h, this resulted in formation of ZnCl₂ nanocolloid.

The nanoparticles were then collected by centrifugation at 2000 rpm for 15 minutes and further purification was made in ultrasonic bath. The resultant product was finally dried at 120°C for 2h. ^[15]

Characterization of ZnS nanoparticles

The prepared sample was subjected to characterization by X-ray diffraction (XRD) (Model D8, Bruker AXS) to determine the phase purity and average particle size of the sample, using CuK α radiation at 1.5409Å ($2\theta = 10^0-70^0$, scan speed = 0.2 s/step, increment = 0.02, operating voltage = 40 kV and operating current = 40 mA). The nanophase was identified by comparing peak positions and intensities (finger print method) ^[16].

To investigate the morphological structure of sample surfaces, surface textures were examined by field emission scanning electron micrography (FESEM) and energy dispersion X-ray fluorescence spectroscopy (EDAX) (JSM6700F JEOL LTD, Tokyo, Japan), was also carried out to ascertain the composition.

Method followed for germination of seeds

The seed germination experiment was carried out with three sets, each set being taken in a Petri plate containing 12 ml of water as basal medium without any growth regulators. First set of Petri plate was considered as the control which consist of basal medium only. Second set was basal medium + 1 ml of ZNPs (10 mg/ml) and 3rd set with 1 ml (20 mg/ml) and of ZNPs. Fifty seeds were placed in each Petri plate and observed for germination.

RESULTS

X-Ray Diffraction (XRD) analysis

From the XRD results, it is clear that pure ZnS nanoparticles were obtained in powder form. The broadened peaks in the XRD pattern indicated the formation of ZnS nanocrystals with small crystallites. The three diffraction peaks at 2θ values of 28.978° , 47.62° , 56.65° corresponding to the (111), (220) and (311) diffraction planes, respectively of the spherical nanocrystalline structure of ZnS were observed. These values were very close to those reported by Jia Xiang Yang *et al.*^[16].

The average crystallite size (D) was calculated from the full-width at half-maximum (FWHM) of the most intense peak of the (111) plane of ZnS nanoparticles using the Debye-Scherrer formula for spherical particles [Eq. (1)].

$$D = 0.89\lambda / (\beta \cos \theta) \quad (1)$$

Where λ is the wavelength (Cu $K\alpha$), β is the full width at the half-maximum of the ZnS nanoparticles and θ is the diffraction angle.

From this equation the average particle size was estimated to be 29 nm which was also supported through FESEM.

FESEM analysis and EDAX study

Fig 1 shows the FESEM results of as prepared ZnS nanoparticles. It is seen that the ZnS nanoparticles are homogenously dispersed and almost spherically shaped with an average diameter of about 10-30 nm. From the EDAX result the composition of the prepared sample could be obtained which was about 73.55% of Zn⁺ ion and about 26.45% S ion by mass present in the sample.

Differences in germination time

Sprouting of seeds was observed from the 5th hr onwards in ZNPs treated plates, however, the sprouting of seeds was observed after 6th hr in the plates that had no ZNPs (control). All seeds treated with ZNPs completed the germination within 4.5 to 7 hrs. But, 24 to 30hr were required for germination in the control plates. The seedlings grown in the plates supplemented with ZNPs increased in length much faster compared to the control seedlings (Fig.2). Maximum height (12.8 cm) of the seedlings was found in the 3rd set which had 20 mg/ml ZNPs, whereas the seedlings in the control plate were much shorter in height than those with ZNPs. All ZNPs treated seedlings attained maximum growth from 11 to 12.8cm within 3 to 7 days. However, the seedlings in the control plates failed to attain the height above 8.1cm.

Table 1: Effect of zinc sulphide nanoparticles on the germination of seeds of *Vigna radiata*.

Concentration of ZNPs	Initial time of germination	% of seed germinated
Control(with no ZNPs)	6 th hr onward	40
10 mg/ml	5 th hr onward	60
20 mg/ml	4.5 th hr onward	70

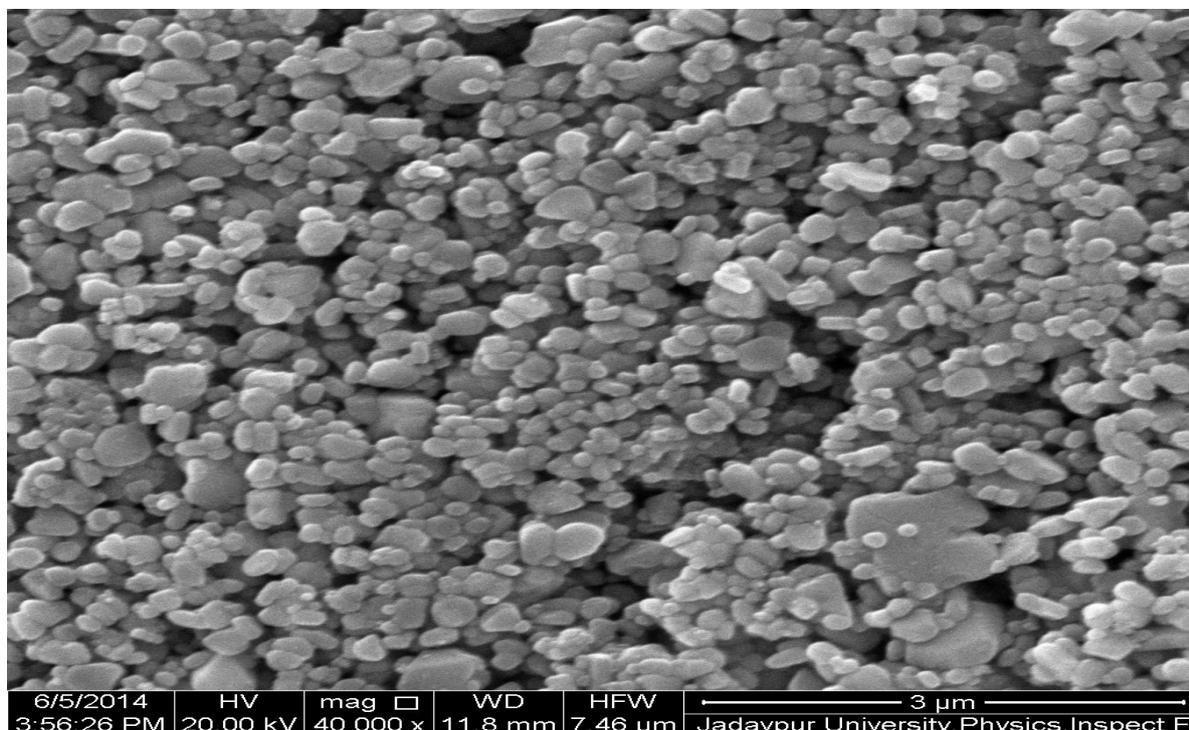


Fig 1: Scanning Electron Micrograph of ZnS Nanoparticles.

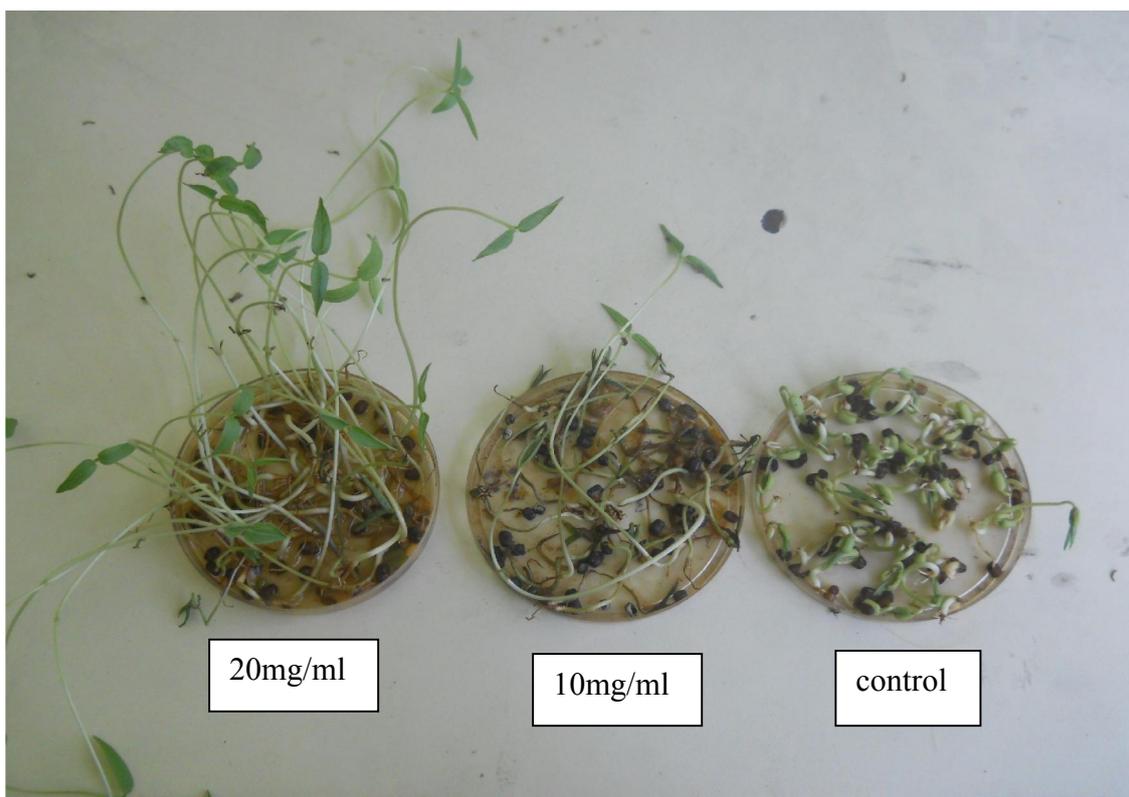


Fig 2: Growth of plants on Petri plate after 3 days. Plates are arranged in the order of decreasing ZNP concentration from the left.

DISCUSSION

The present study clearly indicates that ZnS nanostructures could be synthesized by a simple aqueous chemical method using pure aqueous route resulting in primary particle sizes of 29 nm. This particle size was calculated from Debye –Scherrer formula. FESEM image was used to study the morphology of the synthesized nanoparticles.

The seeds placed in petri plates containing ZNPs revealed greater number with respect to germination, as opposed to the seeds in the control plate which had only distilled water (Table-1). The reason could be that the ZNPs can penetrate through seed coat and may even activate the embryo. Khodakovskaya *et al.* ^[11] observed that the carbon nanotubes could effectively penetrate through seed coat, and influence seed germination. Exposure of tomato seeds to Carbon nanotubes (CNTs) resulted in enhanced seed germination and growth rate ^[10]. Mazumdar and Ahmed ^[17] reported that higher concentrations of chemically synthesized silver nano-particles were toxic to the seedlings of *Oryza sativa* in Hoagland's nutrient solution. The chemically synthesized ZNPs of 20 mg/ml amount was found to be an optimum concentration among the selected concentrations to enhance the maximum growth in

seedlings of *Vigna radiata* germinated in *in vitro* conditions with the help of plain distilled water. The reason could be that the ZNPs generated new pores on seed coats during penetration which may help to influx the nutrients inside the seed or ZNPs may carry the nutrients alongwith which may lead to rapid germination and increased growth rate. The results of the present study may be helpful to improve the % of seed germination and seedling growth in seeds especially in dormant condition. By using this technique it can increase the amplification of plants particularly important for lentil consumption. The increased seedling growth rate may possibly be due to the enhancement of intake of water and trace nutrients uptake by the treated seeds. Also ZNPs may have acted as micronutrients in the process of germination.

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