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THE PATENT OFFICE
पेटेंट प्रमाणपत्र
PATENT CERTIFICATE
(Rule 74 Of The Patents Rules)

पेटेंट सं. / Patent No.	:	304588
आवेदन सं. / Application No.	:	3586/CHE/2011
फाइल करने की तारीख / Date of Filing	:	19/10/2011
पेटेंटी / Patentee	:	INDIAN INSTITUTE OF TECHNOLOGY

प्रमाणित किया जाता है कि पेटेंटी को उपरोक्त आवेदन में यथाप्रकटित APPLICATIONS OF NANOSCALE ZNO IN PEANUT CROP नामक आविष्कार के लिए, पेटेंट अधिनियम, १९७० के उपबंधों के अनुसार आज तारीख 19th day of October 2011 से बीस वर्ष की अवधि के लिए पेटेंट अनुदत्त किया गया है।

It is hereby certified that a patent has been granted to the patentee for an invention entitled APPLICATIONS OF NANOSCALE ZNO IN PEANUT CROP as disclosed in the above mentioned application for the term of 20 years from the 19th day of October 2011 in accordance with the provisions of the Patents Act,1970.

एकस्व कार्यालय THE PATENT OFFICE GOVT.OF INDIA भारत सरकार

अनुदान की तारीख : 18/12/2018
Date of Grant :

पेटेंट नियंत्रक
Controller of Patent

टिप्पणी - इस पेटेंट के नवीकरण के लिए फीस, यदि इसे बनाए रखा जाना है, **19th day of October 2013**को और उसके पश्चात प्रत्येक वर्ष मे उसी दिन देय होगी।

Note. - The fees for renewal of this patent, if it is to be maintained will fall / has fallen due on **19th day of October 2013** and on the same day in every year thereafter.

FORM 2

THE PATENTS ACT, 1970

(39 OF 1970)

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The Patents Rules, 2003

COMPLETE SPECIFICATION

(Refer section 10 and rule 13)

TITLE OF THE INVENTION:

METHOD FOR PREPARING NANOSCALE MONODISPERSE ZNO PARTICLES

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3. Preamble to the Description

COMPLETE SPECIFICATION

The following specification particularly describes the invention and the manner in which is to be performed.

COMPLETE SPECIFICATION

TITLE OF THE INVENTION

METHOD FOR PREPARING NANOSCALE MONODISPERSE ZNO PARTICLES

FIELD OF INVENTION

[001] This invention discloses a method for preparing zinc oxide nanoparticles from zinc oxalate. The nanoparticles are suspended in water as a liquid suspension resulting in spherical ZnO particles with uniform size distribution, high crystallinity, as well as favorable surface chemistry.

PRIOR ART

[002] Zinc (Zn) is an essential mineral for a wide variety of physiological and biochemical processes in all organisms. The deficiency of Zn can cause severe health hazards in humans and Zn supplementation is particularly required in children to achieve reduction of morbidity and mortality which is often difficult to achieve in developing countries. (Bhutta, Z. A., Black, R. E., Brown, K. H., Gardner, J. M., Gore, S., Hidayat, A., Khatun, F., Martorell, R, Ninh, N. X., Penny, M. E., Rosado, J. L., Roy, S. K., Ruel, M., Sazawal, S., Shankar, A. 1999. Prevention of diarrhea and pneumonia by zinc supplementation in children in developing countries: pooled analysis of randomized controlled trials. Journal of Pediatrics 135:689-697, Sazawal S., Black, R. E., Menon, V. P., Dhingra, P., Caulfield, L. E., Dhingra, U., Bagati, A. 2001. Zinc supplementation in infants born small for gestational age reduces mortality: a prospective randomized controlled trial. Pediatrics 108:1280-1286.) Plants also greatly suffer from the deficiency of Zn. Zn being an essential component of various enzyme systems for energy production, protein synthesis, and growth regulation, its deficiency in plants will be demonstrated by delayed maturity. This suggests that there is a need for a constant supply of available Zn for optimum growth in plants. The short internodes and a decrease in leaf size and delayed maturity are the most common symptoms of Zn¬ deficient plants. For humans, Zn supplementation through food is a powerful therapeutic tool in managing a long list of illnesses. Hence, it is suggested that a considerable amount of research should be devoted to develop technologies for enhanced uptake and accumulation of micronutrients, in particular Zn, in edible plant parts.

[003] Zinc occurs in a wide variety of foods, but is relatively high in nuts, legumes and whole grain cereals. Peanut or groundnut (Arachis hypogaea L) is one of the top ten foods rich in Zn [100 g of oil-roasted peanuts will provide 6.6 mg (44% RDA) of Zn]. Peanut has high protein and high oil content. Hence, as an important oil and legume food crop, it is grown in 108 countries around the world. Zinc deficiency in groundnut causes irregular mottling and interveinal chlorosis in leaves. In India, about 50% of groundnut soils show Zn deficiencies causing considerable yield loss (Singh, A. L. 1999. Mineral nutrition of groundnut. In: Hemantharajan, A (Ed.). Advances in plant physiology. 161-200, Scientific publications (India) Jodhpur.; Singh, A. L., Basu, M. S., Singh, N. B. 2004. Mineral disorders of groundnut. National Research centre for groundnut Junagadh, India, 85, ICAR Publications, ICAR, New Delhi). Thus there is a need to invent novel stratergies for the effective delivery of Zn nutrition which plays an important role in stepping up the productivity of groundnut. The increase in productivity of peanut can be an important step forward in helping to reduce the Zn deficiency and the related hazards to human kind. Though India has largest area under cultivation for groundnut (about 8 million ha of land), the low productivity is mainly due to the fact that the crop is mostly grown in rain-fed, low fertility soils.

[004] Peanuts fall into four basic types: Runner, Virginia, Spanish and Valencia. Each of these peanuts is distinctive in size and flavour. Demand for peanuts has been steady with a growing range of value added peanut products (peanut butter, milk, flour in jelly, confectionary, cookies and candies). Increases in price of these peanut products are reported every time with fall in peanut production. There is a lucrative market for peanut worldwide. Though India has largest area under cultivation for peanut (about 8 million ha of land), the low productivity is mainly due to the fact that the crop is mostly grown in rain-fed, low fertility soils.

[005] Higher plants generally absorb Zn as a divalent cation (Zn^{2+}) which acts either as the metal component of enzymes or as a functional, structural or a regulatory co-factor of a large number of enzymes. Most of the research conducted on the micronutrient nutrition of peanut crop deals with the application of Zn along with other nutrients to influence the growth, yield and productivity (Gowda, G. N., Shivaraj, B. Gowda, A. 1994. Effect of zinc and molybdenum application on yield and uptake of zinc by groundnut. Journal of Research, Andhra Pradesh Agricultural University 22: 40-42.; Majumdar, B., Venkatesh, M. S., Lal, B., Kumar, K., Singh, C. S. 2001. Effect of phosphorous and zinc nutrition on groundnut in an acid hapludalf of

Meghalaya. Annales Agriculturae Research New Series 22:354-359, Geeta, K. N., Shankar, A. G, Shiva Shankar, K. 1996. Effect of molybdenum, zinc and calcium on productivity of groundnut (Arachis hypogaea Gaertu). Journal of Oilseeds Research 13:167-172).

[006] The methods that have been in the art to deliver Zn to plants use Zn in the form of Zn salts and chelates. Because of the high solubility of Zn salts in water, a Zn salt, such as Zn sulphate or a chelated form, delivers Zn through the roots or foliage systems of a plant. These are the preferred and accepted methods for increasing the Zn levels within a plant. Hence farmers are widely using sulphates and chelated ZnSO4, (Zn with ethylenediamine tetraacetic acid, EDTA) for soil and foliar applications. However, the efficacy of such methods is low and high concentrations of Zn are needed to obtain the desired results. One disadvantage with these forms of Zn is that they are very soluble in water and tend to wash off foliage with dew and precipitation. Furthermore, Zn salts are more difficult and expensive to formulate in combination with other agents or fertilizers. The prior art /patents dealing with this aspect are given below:

a) United States Patent US 3130034, Zinc containing liquid fertilizer.
b) United States Patent 3620708, Liquid fertilizers including soluble zinc from zinc oxide.
c) United States Patent Application 3930832, Inhibition of corrosive action of zinc-containing fertilizer solutions.
d) United States Patent 4025330, Zinc-containing foliar spray.
e) United States Patent 5667795, Pesticidal micronutrient compositions containing zinc oxide.

[007] Despite these earlier efforts, there is a need for a method which is less expensive to formulate and to deliVer Zn more effectively to plants. In the United States Patent 3130034, one method of Zn containing liquid fertilizer wherein an aqueous solution formulated by incorporating $ZnSO_4$ in aqueous ammonia was described to provide a solution with a desirable salt out temperature. Unfortunately, aqueous solutions of $ZnSO_4$ corrode milled steel and form large quantities of scale and rust in process tanks and equipment. Hence, in another method, the formulation consisted of water, ZnO, phosphorous pentoxide, and ammonia to create ammonia based fertilizer in solution providing nitrogen, phosphorous and solubilised Zn.

[008] It is well understood that the particle size may affect agronomic effectiveness of Zn fertilizers. Decreased particle size results in increased number of particles per unit weight of applied Zn. Decreased particle size also increases the specific surface area of a fertilizer, which

should increase the dissolution rate of fertilizers with low solubility in water such as ZnO (Mortvedt, J. J., 1992. Crop response to level of water soluble Zn in granular Zn fertilizers, Fertilizer Research 33:249-255). Granular $ZnSO_4$ (1.4 to 2 mm) was somewhat less effective than fine $ZnSO_4$ (0.8 to 1.2 mm). Gradual increase in Zn uptake was observed with decreasing granule size and only the powder form produced plants with Zn concentrations in the sufficient range. Since granules of 1.5 mm weighs less than granules of 2.0 or 2.5 mm, smaller granules were used for the same weight, resulting in a better distribution of Zn, higher surface area of contact of Zn fertilizer resulted in better Zn uptake (Liscano, J. F., Wilson, C. E., Norman, R. J. Jr., Slaton, N. A. 2000. Zinc availability to rice from seven granular fertilizers. AAES Research Bulletin 963:1-31). Therefore, ample work has been done and emphasis was made on the particle size to increase the efficiency of the fertilizers for better uptake and higher yields. To solve the problem of particle size, recent advances in materials science and chemistry have produced mastery in nanoparticle technology, with wide applications in the field of agriculture. Nanotechnology has the potential to revolutionize the food and agricultural industry with new tools for rapid disease detection, enhancing the ability of plants to absorb nutrients, etc. Extensive reports on the use of nanoparticles and their effectiveness and/or the biological effects on higher plants are available in open literature and few prior arts are reported below.

a) WIPO Patent Application W02011059507, Method of using carbon nanotubes to affect seed germination and plant growth.
b) United States Patent Application 20110094277, Nano-scale urea particles and methods of making and using the particles.
c) United States Patent Application 20110000411, Biologically active multifuctional nanochips and method of application thereof for production of high-quality seed.
d) United States Patent Application 20100139347, Nano-composite super absorbent containing fertilizer nutrients used in agriculture.
e) WIPO Patent Application WO/2010/068275, Silica based antibacterial and antifungal nanoformulation United States Patent Application 20100326153, Preparation of a nano long-acting selenium fertilizer.
f) Ma, Y., Kuang, L., He. X., Bai, W., Ding, Y., Zhang, Z., Zhao, Y., Chai, Z. 2010. Effects of rare earth oxide nanoparticles on root elongation of plants. Chemosphere 78:273-9.

g) United States Patent Application 20090075818, Nanosilver for preservation and treatment of diseases in agriculture field.

h) Seeger, E. M., Baun, A., Kastner, M., Trapp, S., 2009. Insignificant acute toxicity of TiO2 nanoparticles to Willow trees, Journal of Soils and Sediments 9:46-53.

i) Doshi, R., Braida, W., Christodoulatos, C., Wazne, M., O'connor, G., 2008. Nano aluminium: transport through sand columns and environmental effects on plant and soil communities. Environmental Research 106:296-303.

j) Zhu, H., Han, J., Xiao, J.Q., Jin, Y., 2008. Uptake, translocation and accumulation of manufactured iron oxide nanoparticles by pumpkin plants. Journal of Environmental Monitoring 10:713-717.

k) Racuciu, M., Creanga, D., 2007. TMA-OH coated magnetic nanoparticles internalized in vegetal tissues. Romanian Journal of Physics 52:395-402.

l) WIPO Patent Application WO/2006/049379, Composition for controlling pathogenic microorganisms in plants.

m) Nel, A., Xia, T., Madler, L., Li, N., 2006. Toxic potential of materials at the nanolevel. Science 311:622-627.

n) Yang, F., Hong, F. S., You, W. J., Liu, C., Gao, F. Q., Wu, C., Yang, P. 2006. Influences of nano-anatase TiO2 on the nitrogen metabolism of growing spinach. Biological Trace Element Research 110:179-190.

o) Hong, F. S., Yang, F., Ma, Z. N., Zhou, J., Liu, C., Wu, C., Yang, P., 2005. Influences of nano-TiO2 on the chloroplast ageing of spinach under light. Biological Trace Element Research 104:249-260.

p) Yang L., Watts, D. J. 2005. Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles. Toxicology Letters 158:122-132.

q) Lu, C. M., Zhang, C. Y., Wen, J. Q., Wu, G. R., Tao, M. X. 2002. Research of the effect of nanometer materials on germination and growth enhancement of glycine max and its mechanism. Soya Bean Science 21:168-172.

r) Lin, D., Xing, B., 2007. Phytotoxicity of nanoparticles: Inhibition of seed germination and root growth. Environmental Pollution 150:243-250.

s) Zhang, L., Hong, F., Lu, S., Liu, C., 2005. Effect of nano TiO_2 on strength of naturally aged seeds and growth of spinach. Biological Trace Element Research 105:83-91.

[009] A few important highlights from the work reported in the prior art is as follows: Different plants have different response to the same nanoparticles. Phytotoxicity of nanoparticles on plants was reported at concentrations greater than 2000 ppm. Properties of engineered nanoparticles depend on the size, shape, surface functionalisation, etc. Carbon-based and metal-based nanoparticles are most commonly engineered and are often studied. However, research on ZnO nanoparticles has been voluminous in prior art although most of those are not of relevance to agriculture.

[010] ZnO nanomaterial is unique with a long history of use in many researches including agriculture. The synthesis of ZnO nanoparticles has been attempted by various physical and chemical methods. The effect of surface modification on the size, structure, morphology, and other properties of ZnO nanoparticles are demonstrated. The biosynthesis and characterization of ZnO have been attempted in natural sources like microbes, algae and/or plants (Brayner, R., Dahoumane, S. A., Yprmian, C., Djediat, C., Meyer, M., Cout, A., Fivet, F. 2010. ZnO Nanoparticles: synthesis, characterization, and ecotoxicological studies. Langmuir 26:6522-6528). Numerous methods of phytoextraction/ hyper accumulating plant mediated biosynthesis of ZnO nanoparticles are reported (Qu, J., Yuan, X., Wang, X., Shao, P. 2011 Zinc accumulation and synthesis of ZnO nanoparticles using Physalis alkekengi L. Environmental Pollution 159:1783-1788; Qu, J., Luo, C., Hou, J. 2011. Synthesis of ZnO nanoparticles from Zn-hyperaccumulator (Sedum alfredii Hance) plants, Micro & Nano Letters, IET 6:174-176). The antimicrobial property of ZnO nanoparticles not only finds use in food processing and health sector but also in producing antimicrobial textiles (Rajendra, R., Balakumar, C., Ahammed, H. A. M., Jayakumar, S., Vaideki, K., Rajesh, E. 2010. Use of ZnO nanoparticles for production of antimicrobial textiles, International Journal of Engineering Science and Technology 2:202-208).

[011] Nanomaterials are proposed to be the materials of choice to solve the problem of particle size that may affect agronomic effectiveness of Zn in crops. The methodologies adopted in most of the previous methods for ZnO nanomaterial growth and formations have specific applicability based on specific need. Improvements in the method of making ZnO nanoparticle for application in plants are relatively need based. In several cases, the application of ZnO nanoparticles above a specific concentration was found to have detrimental effects on the plant growth. Hence, there exist a need to formulate strategies to create ZnO-based nanomaterials which can be applied to crops at optimum concentrations for better uptake and higher transfer inside the plant, thereby

increasing crop yield besides increasing its bio-availability in plant parts. Peanut, being one of the most important oil seed cum legume plants having large area under cultivation all over the world, especially in India, was chosen as the model plant system for this invention.

[012] Considering all aspects described above, we have invented a method where custom made nanoscale ZnO particles can be used for application in peanut that essentially consists of application methods for enhancing Zn concentrations in seed and/or other parts of the plant. Owing to a high surface area to volume ratio, the nanofertilizers can be used for Zn nutrition in plants. The evaluation to assess the potential of nanomaterials in plants by accurate in vitro/in vivo screening assays is a highly desirable goal.

[013] This invention was taken up to demonstrate the promotory or inhibitory effects of various concentrations of custom made nanoscale ZnO particles on growth, development and final yield of peanut.

[014] In India, Andhra Pradesh is second largest peanut producing state after Gujarat. Peanut seeds of variety K-134 were procured from Agricultural Research Station, Kadiri, Acharya N. G. Ranga Agricultural University, Andhra Pradesh, India.

SUMMARY OF THE INVENTION

[015] The present invention relates to a method for preparing nanoscale monodisperse spherical ZnO particles of size up to 25 nm. The method comprises zinc oxalate prepared from solutions of zinc acetate and oxalic acid or a combination thereof. The zinc oxalate is decomposed at a temperature range of 300-600 °C and suspended in water as a liquid suspension which results in spherical ZnO particles with uniform size distribution, high crystallinity, as well as favorable surface chemistry . The unique surface chemistry, size, and crystallinity of the ZnO NPs leads to enhanced uptake and Zn delivery in many industrial applications.

[016] In one embodiment, the present invention illustrates the synthesis of spherical ZnO particles which is uniform in size distribution, high crystallinity, as well as favorable surface chemistry. This methodology results in comparatively monodisperse spherical ZnO particles of average diameter 25 nm. The stable dispersion of ultra-small nanocrystalline ZnO NPs, in turn, results in the facile application process and improved uptake by the plants leading to enhanced productivity. The hydroxy surface layer on ZnO nanoparticle plays a significant role in controlling the surface charge, which can be further modified by various environmental factors

including pH. It is known that plant roots readily uptake positively charged nanoparticles. This unique surface characteristic of ZnO nanoparticles improve their interaction with the plasma membrane of the plant roots and facilitate their translocation into the plant shoot, which could potentially improve the productivity.

DRAWINGS AND TABULATED DATA

Figure captions

[017] Figure 1: Large area TEM image of ZnO nanoparticles. Inset shows the high resolution image of a single particle.

[018] Figure 2: (A) SEM image of the peanut seed embryo after soaking in nanoscale ZnO (1000 ppm) for 3 h. (B, C, D) EDAX images of the region in A using Zn La, C Ka and N Ka lines. (E) EDAX spectrum from the region in A.

[019] Figure 3: Photographs of the seeds showing differences in germination and root growth (A) after three days and (B) nine days after the treatment.

[020] Figure 4: (a) Higher root growth of peanut plant after nanoscale ZnO treatment (1000 ppm). The plants were uprooted after 110 days. (b) Pot culture experiment showing higher plant growth after nanoscale ZnO treatment (1000 ppm), after 110 days.

[021] Figure 5: Bar chart showing the effect of nanoscale ZnO (N) and bulk $ZnSO_4$ (B) concentrations on peanut root stem growth and pod yield.

[022] Figure 6: Photograph showing the effect of foliar application of lower dosage of nanoscale ZnO on the pbd yield. A and B) nanoscale ZnO @ 2 g/ 15 I and C) control.

[030] Table 1: Effect of nanoscale ZnO and bulk $ZnSO_4$ on peanut mean germination and vigor.

[023] Table 2: Effect of nanoscale ZnO and bulk $ZnSO_4$ on peanut mean plant growth, flowering and leaf chlorophyll content.

[024] Table 3: Effect of nanoscale ZnO and bulk $ZnSO_4$ on mean root growth, shoot growth, dry weight and pod yield in peanut.

[025] Table 4: Response of peanut to application of nanoscale ZnO.

[026] Table 5: Effect of nanoscale Zn on yield and yield attributes of peanut (Rabi season 2008-09).

[027] Table 6: Effect of nanoscale Zn on yield and yield attributes of peanut (Rabi season 2009-10).

[028] Table 7: Effect of nanoscale ZnO on uptake of Zn by leaf and kernel of peanut.

DESCRIPTION WITH REFERENCE TO DRAWINGS AND TABULATED DATA

[029] The present invention relates to a method for preparing nanoscale monodisperse spherical ZnO particles of size up to 25 nm. The method comprises zinc oxalate prepared from solutions of zinc acetate and oxalic acid or a combination thereof. The zinc oxalate is decomposed at a temperature range of 300-600 °C by suspending it in water as a liquid suspension which results in spherical ZnO particles with uniform size distribution, high crystallinity, as well as favorable surface chemistry.

Experimental methods,

Material characterization

ZnO nanoparticles characterization

[030] The ZnO nanoparticles used in this invention was characterised using high resolution transmission electron microscopy (HRTEM, JEOL 3010), scanning electron microscopy (SEM, FEI Quanta 200) and energy dispersive analysis of X-rays (EDAX, FEI Quanta 200). For SEM and EDAX measurements, the ZnO nanoparticles prepared as mentioned in the experimental section was re-suspended in water by sonication for 10 min and drop castedon an indium tin oxide (ITO) conducting glass plates and dried. Thin sections of the seed were stuck on conducting carbon tape and EDAX measurements were carried out. The TEM samples were prepared by drop casting the suspensions on carbon coated Cu grids. The post harvest leaf and kernel samples were analyzed to estimate the Zn content by using Atomic Absorption Spectrophotometry (AAS).

Example 1

[031] This embodiment comprises of the synthesis of extremely small ZnO nanoparticles via a modified oxalate decomposition technique and their subsequent applications in developing highly efficient peanut seeds. In a typical synthesis, zinc oxalate was prepared by mixing equimolar (0.2 M) solutions of zinc acetate and oxalic acid. The resultant precipitate was collected and rinsed extensively with double deionized water (DI-water) and dried in air. The

oxalate was then ground and decomposed in air by placing it in a pre-heated furnace at 500 °C for 45 minutes. The resultant nanoparticles are suspended in water as a liquid suspension. This approach also offers extremely small nano magnesium oxide (3-7 nm) in relation to the existing combustion based synthesis methods. ZnO nanoparticles of mean crystallite size of 25 nm diameter were synthesized and were used in this invention.

Example 2

[032] In order to evaluate nanoscale ZnO on peanut germination, the following protocol was adopted. Chelated bulk $ZnSO_4$ was used as a reference Zn source. Because bulk ZnO will not dissolve in water, we used chelated $ZnSO_4$ as the reference with equal Zn content. This material was suspended directly in deionised water and dispersed by ultrasonic vibration (100 W, 40 KHz) for 30 min. Magnetic bars were placed in the suspensions for stirring before use to avoid aggregation of the particles. Both bulk (chelated) $ZnSO_4$ and nanoscale ZnO suspensions were prepared at concentrations of 400, 1000 and 2000 ppm (concentrations referred to in terms of Zn content). Five peanut seeds were soaked in 100 mL of these solutions/suspensions of both bulk $ZnSO_4$ and nanoscale ZnO for 3 h. Four replicates were maintained. The suspensions were labelled such that B and N refer to bulk $ZnSO_4$ and nanoscale ZnO, respectively.

For example, 400B and 400N referred to suspensions of 400 ppm bulk $ZnSO_4$ and nanoscale ZnO, respectively. The nanoscale suspensions, as expected, 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.

Example 3

[033] With one set of treated seeds (4 replicates) explained in the Example 2, lab experiments were conducted to determine the effect of treatment on seed germination and seedling vigour index. Treated peanut seeds were shade-dried for 1 h. Then the seeds were placed in a' Petri dish (100 mm x15 mm) with one piece of sterilized filter paper and 5 mL of water was added (as per the recommendations of the International Seed Testing Association, 1976). Petri dishes were covered and placed in an incubator at 26±1 °C for 8 days. Watering was given to all plates. After 8 days, maximum seeds were germinated and developed into normal seedlings. Germination was calculated based on the number of seeds germinated in a Petri plate having 5 seeds and expressed as germination percentage. "Seedling Vigour Index" (SVI) was calculated by the formula

described by Abdul-Baki and Anderson (Abdul-Baki, A. A., Anderson, J. D. 1973. Vigor determination in soybean seed by multiple criteria. Crop Science 13:630¬633).

Seed Vigour Index = Germination % x (root length (cm) + shoot length(cm))

Example 4

[034] In this example, pot culture experiments were carried out with a set of treated peanut seeds (4 replicates) explained in Example 2, sown in pots (20 cm x 40 cm) filled with equal quantity of soil and watered to field capacity. Proper care was taken to use similar soil in all the pot's to minimize soil heterogeneity effects. After germination, one plant per pot was maintained throughout. Proper agronomic and plant protection management was done to all the treated plants for their maximum growth expression. The following data were collected on all the plants of four replications.

a) Plant height was measured from ground node to shoot growing apex and expressed in cm before harvest.
b) Days to flowering were calculated based on the days taken from sowing to the appearance of first flower.
c) The procedure developed by Witham et al., was followed for estimation of chlorophyll content of leaves (Witham, F. H., Blaydes, D. F., Devlin, R. M. 1971. Chlorophyll absorption spectrum and quantitative determinations, "Experiments in Plant Physiology". Von Nostra and Ren FOLD Company New York. pp 55-56.).

Example 5

[035] After 110 days from sowing, plants were uprooted gently along with the whole soil mass. Plant with whole root was recovered by spraying fine water on the soil mass. Roots were separated and used for recording the parameters. Similarly, matured, filled and unfilled pods were dried to the moisture level of 12% and dry weight per plant was recorded.

[036] Roots were thoroughly washed and their volume was measured by water replacement method which was expressed in mL and total length was measured and expressed in cm. Then the roots were dried for two days at 80 00 in an oven and dry weight was taken and expressed in grams.

Example 6

[037] The field experiment was conducted with peanut (Narayani) during Rabi seasons (2008-'09, 2009-'10). The Zn application was done in two ways viz, foliar spray (in the field) and seed treatment (pot culture). The experiment was laid out in randomised block design replicated 7

times. The gross plot size was 4x5 m2. Three treatments viz., Ti: NPK (30-40-50), T2: NPK + chelated Zn @30 g/15 L Foliar spray (at 35 days and 70 days), and T3: NPK + nanoscale ZnO (size 25 nm) @ 2 g /15 I foliar spray (at 35 days and 70 days) were impo'sed. The initial soil (red sandy loam) parameters were pH: 6.85, E.C. (dSm-1): 0.132, O.C. %: 0.485, available P205: 14.43 kg ha-1, K20: 172 kg ha-1, Zn: 0.74 ppm, Cu: 1.55 ppm, Fe: 9.93 ppm and Mn: 28.06 ppm. Plant physiological parameters viz., plant height, and number of branches was recorded in all the treatments.

Example 7

[038] Each treatment was conducted with seven replicates and the results were presented as mean ± standard error (SE). The statistical analysis of experimental data utilized the ANOVA program. Each experimental value was compared to its corresponding control. Statistical significance was accepted when the probability of the result assuming the null hypothesis (p) is less than 0.05 (level of probability).

ZnO nanoparticle characterization

[039] The HRTEM image (Figure 1) shows ZnO nanoparticles with mean particle diameter of 25 nm and they looked slightly aggregated as there were no protecting ligands on the surface. The particles are crystalline as revealed by the high magnification image and the lattice of ZnO is clearly seen.

[040] The uptake of Zn by the seeds was confirmed by SEM-EDAX measurements (Figure 2). SEM of thin sections of the peanut embryo was examined by sectioning the seed. Although the concentration of Zn was low as expected, it could be observed in EDAX spectra and the EDAX images confirmed the presence of higher amounts of Zn in regions where C and N concentrations are higher in the seeds treated with nanoscale ZnO. The leaf and kernel samples were analyzed post harvest using atomic absorption spectrophotometer (AAS). This also confirmed the presence of Zn (Table 7).

Seed germination and 'seedling vigor'

[041] Peanut seeds responded variably towards the treatment at various concentrations of both bulk ZnSO4 (Chelated) and nanoscale ZnO particles. Seed treated with 1000 ppm nanoscale ZnO recorded significant germination (100%) and seedling vigour index (1701.3). Root growth was also very good as can be observed from the picture (Figure 3). The results from the bulk ZnSO4

treated seeds were not promising (Table 1). Among the different ZnO concentrations, 1000 ppm showed the maximum seedling vigour index but a concentration of 2000 ppm showed a decrease. Such inhibitory effects of nanoparticles were also reported by Lin and Xing (Lin, D., and B. Xing. 2007. Phytotoxicity of nanoparticles: Inhibition of seed germination and root growth. Environmental Pollution 150:243-250) on radish, rape and rye grass. However, performance of the bulk material is better than the control.

[042] Nanoscale ZnO described in this embodiment showed large root growth of seedling compared to bulk ZnSO4 and control. Such promotory effect of nanoscale SiO2 and TiO2 on germination was reported in soya bean (Lu, C. M., C. Y. Zhang., J. Q. Wen., G. R. Wu., and M. X. Tao. 2002. Research of the effect of Nanometer materials on germination and growth enhancement of Glycine max and its mechanism. Soya Bean Science 21:168-172), in which authors noticed increased nitrate reductase enzyme activity and enhanced antioxidant system. Plant growth, in terms of plant height was significantly increased with 400 and 1000 ppm of nanoscale ZnO compared to control and the respective bulk ZnSO4 concentrations (Table 2). Seeds treated with 1000 ppm concentration of nanoscale ZnO recorded highest plant growth (15.4 cm) due to extended inter-nodal length. Such increase can be ascribed to higher precursor activity of nanoscale ZriO in auxin production (Kobayashi, Y., and S. Mizutani. 1970. Studies on the wilting treatment of corn plant: The influence of the artificial auxin control in nodes on the behaviour of rooting. Proceedings of the Crop Science Society of Japan 39:213-220). Similarly 1000 ppm nanoscale ZnO produced early flowers compared to control and bulk ZnSO4. Such effects can be due to higher seedling vigour and early vegetative growth. Nanoscale ZnO increased leaf chlorophyll content irrespective of concentrations compared to bulk ZnSO4 and control. Nanoscale ZnO at 1000 ppm recorded the highest leaf chlorophyll content (1.97 mg/g). Higher chlorophyll accumulation may be due to complementary effect of other inherent nutrients like magnesium, iron and sulphur. Similar results were observed by Zhang et al., 2005 (Zhang, L., F. Hong., S. Lu., and C. Liu. 2005. Effect of nano TiO2 on strength of naturally aged seeds and growth of spinach. Biological Trace Element Research 105:83-91) when Spinacia oleracea seeds were treated with nanoscale TiO2 particles. An increase of germination rate and the vigor indices was noted at 0.25-4% nanoscale TiO2 treatment. During the growth period, the plant dry weight increased. These results confirmed that the physiological effects were related to the nanometre sized particles.

Root growth and yield

[042] The plants cultured as per the present invention were harvested after 110 days from sowing. The results revealed the promotory effect of nanoscale ZnO at optimum concentrations and inhibitory effect at high concentrations on root and shoot growth (Figure 4) and pod yield (Table 3). Nanoscale ZnO at 1000 ppm proved to be effective in improving both root volume and root dry weight, as it was also noticed in the seedling stage (Figure 5). An increase of the shoot/root ratio compared to that of the control was reported by Shah and Belozerova (Shah, V., I. Belozerova. 2009. Influence of metal nanoparticles on the soil microbial community and germination of lettuce seeds. Water, Air and Soil Pollution 97:143-148) while analyzing the influence of metal nanoparticles on germination of Lactuca seeds. Due to its promotory effects on plant growth, pod yields were significantly increased over control and ZnSO4. At higher concentration of nanoscale ZnO, at 2000 ppm, both plant growth and pod yield decreased and these results were in accordance with the reports on radish, rape, corn, lettuce and cucumber by Lin and Xing, 2007 (Lin, D., and B. Xing. 2007. Phytotoxicity of nanoparticles: Inhibition of seed germination and root growth. Environmental Pollution 150:243-250).

Yield and yield attributes

[043] The results revealed that the response of groundnut to lower dose of nanoscale ZnO was highly significant. The dry pod yield of groundnut was greatly influenced by nanoscale ZnO (Figure 6). Figure 6 A and B show increased pod yield upon application of nanoscale ZnO @ 2 g/15 L. The data in Table 4 indicate significant increase in number of pods per plant, number of filled pods per plant, and also plant height with the application of nanoscale ZnO © 2 g/15 L. From the data in Table 5, and Table 6 it is observed that 30.5% and 38.8% higher pod yield was recorded with the application of nanoscale ZnO @2 g/15 L + NPK compared to NPK alone and 29.5% and 26.3% higher pod yield compared to chelated Zn@30 g/15 L + NPK. In general, foliar application of nanoscale ZnO @ 2 g /15 L described in this invention could significantly increase pod yield and shelling percent and other biometric parameters. Figure 6C shows the control experiment at similar conditions showing lower yield.

Discussion of relevant results

[044] Zinc plays a fundamental role in protecting and maintaining structural stability of cell membranes (Welch, R. M., M. J. Webb., and J. F. Loneragan. 1982. Zinc in membrane function and its role in phosphorus toxicity. Scaife A (ed) Proceedings of the Ninth Plant Nutrition

Colloquium. pp. 710-715. Warwick, UK. Wallingford, UK: CAB International; Cakmak, I. 2000. Role of Zn in protecting plant cells from reactive oxygen species. New Phytologist 146:185-205). Zn is used for protein synthesis, membrane function, cell elongation and tolerance to environmental stresses (Cakmak, I. 2000. Role of zinc in protecting plant cells from reactive oxygen species. New Phytologist 146:185¬205). Plants emerging from seeds with low Zn concentration have poor seedling vigor and field establishment On Zn-deficient soils (Yilmaz, A., H. Ekiz., I. Gultekin., B. Torun., H. Barut., S. Karanlik., and I. Cakmak. 1998. Effect of seed zinc content on grain yield and zinc concentration of wheat growth in zinc-deficient calcareous soils. Journal of Plant Nutrition 21:2257-2264). Rengel and Graham (Rengel, Z., and R. D. Graham.1995. Importance of seed Zn content for wheat growth on Zn-deficient soil. I. Vegetative growth, Plant and Soil 173:259-266) reported from pot culture experiments on wheat plants that increasing seed Zn content from 0.25 pg per seed to 0.70 pg per seed significantly improved root and shoot growth under Zn deficiency. Hence it may be concluded that high Zn' content in seed could act as a starter fertilizer. Ajouri et al., (Ajouri, A., H. Asgedom, M. Becker. 2004. Seed priming enhances germination and seedling growth of barley under conditions of P and Zn deficiency. Journal of Plant Nutrition and Soil Science 167:630-636) reported that seed priming with Zn was very effective in improving seed germination and seedling development in barley. Slaton et al., (Slaton, N.A., C. E. Wilson., S. Ntamatungiro., R. J. Norman., D. L. Boothe. 2001. Evaluation of Zn seed treatments for rice. Agronomy Journal 93:152-157) reported that treating rice seeds with Zn greatly increased grain yield and concluded that this type of Zn application method is a very economical alternative to more expensive broadcast Zn fertilizer applications. These results may indicate that high Zn concentration in seeds has very important physiological roles during seed germination and early seedling growth. In the present invention, treating groundnut seeds with nanoscale ZnO particles with a concentration of 1000 ppm has shown significant increment in germination, shoot length, root length and vigour index over other concentrations of the same material and varying concentrations of another material (chelated ZnSO4) tested. Further experiments are required to unravel the factors favouring optimal dose and/or causes for negative effects at higher concentrations of nanoscale ZnO in the seed treatments.

[045] Foliar fertilization is reported to be more effective than soil application. Foliar Zn application significantly increased grain Zn concentrations of wheat grains indicating high

mobility of Zn within plants. Spraying with 0.5% ZnSO4 gave significantly higher peanut pod yield compared to no spraying. However, soil application of 10 kg ha-1 ZnSO4 at sowing gave yield on par with no ZnSO4 application. This indicates that groundnut responds to foliar spray but not to soil application (Channabasavanna, A. S., and R. A. Setty. 1993. Effect of nitrogen, ferrous sulphate and ZnSO4 on groundnut yield in deep black soils. Indian Journal of Agronomy 38:329-330). The effectiveness of various synthetic and natural chelates has been widely investigated (Alvarez, J. M., D. Gonzalez. 2006. Zinc transformations in neutral soil and Zn efficiency in maize fertilization. Journal of Agricultural and Food Chemistry 54:9488-9495; Gonzalez, D., Obrador, A. Alvarez, J. M. 2007. Behaviour of zinc from six organic fertilizers applied to a navy bean crop grown in a calcareous soil. Journal of Agricultural and Food Chemistry 55:7084-7092; Prasad, B., Sinha, M. K. 1981. The relative efficiency of Zn carriers on growth and Zn nutrition of corn. Plant and Soil 62:45-52). Apart from their effectiveness, application of chelates is generally expensive and may result in potential leaching risk because the more mobile the chelate, or the less biodegradable the carrier, the greater the risk of leaching (Gonzalez, D., A. Obrador., Alvarez, J. M. 2007. Behaviour of Zn from six organic fertilizers applied to a navy bean crop grown in a calcareous soil. Journal of Agricultural and Food Chemistry 55:7084-7092). ZnSO4 which is highly soluble can easily be taken up by plants but is known to fall off quickly. The retention time in the plant system is low. Besides poor bioavailability of ZnSO4 nutrients for long periods, with the increased sensitivity of the plants and the surrounding adverse weather conditions like high temperatures, ZnSO4 having high salt index may create scorching effect on the plants. Moreover, the Zn content in the mixture is usually very low (9-12%). Our invention suggests that ZnO in the nanoscale form is absorbed by plants to a larger extent unlike bulk ZnSO4. These particles proved effective in enhancing plant growth, development and yield. A lower dose of foliar application is proved to be significantly productive. The post harvest leaf and kernel samples analysis (Table 7) revealed a significant increment in Zn content in leaves (42%, 29%) and kernels (42%, 36.6%) when supplied with nanoscale ZnO compared to chelated ZnSO4 (in Rabi 2009 and Rabi 2010, respectively). It is reported that nanoscale nutrients at high concentrations are as detrimental as the bulk nutrients. Similar results were observed by Racuciu and Creanga (Racuciu, M., D. Creanga. 2007. TMA-OH coated magnetic nanoparticles internalized in vegetal tissues. Romanian Journal of Physics 52:395-402), when they analyzed the influence of magnetic nanoparticles coated with tetramethylammonium hydroxide on the growth of Zea mays plant in early ontogenetic stages. Small concentrations of aqueous ferrofluid added in culture medium had a stimulating effect on the growth of plantlets while higher concentrations of aqueous ferrofluid induced an inhibitory effect.

[046] The mechanism of foliar uptake pathway for aqueous solutes and water—suspended nanoparticles was well discussed by Thomas Eichert et al., 2008 (Eichert, T., Kurtz, A., Steiner, U., Goldbach, H. E. 2008. Size exclusion limits and lateral heterogeneity of the stomata) foliar uptake pathway for aqueous solutes and water suspended nanoparticles. Physiologia Plantarum 134:151-160) in the context of Allium porrum and Vicia faba (L). The results suggest that the stomatal pathway differ fundamentally from the 'cuticular foliar uptake pathway. Low penetration rates in thick leaves, rapid drying of spray solution, limited translocation within the plant, and leaf damage are the problems of concern (Marschner, H. 1995. Mineral nutrition of Higher plants. 2nd ed, London:Academic Press) and most foliar applied micronutrients are not efficiently transported towards the roots. Concentrated liquid suspensions of ZnO are used for foliar application but their performance is strongly determined by the size range specification of the ZnO particles present in the formulation (Moran, K. 2004. Micronutrient product types and their development proceedings, International Fertilizer Society (York, UK) 545:1-24). Leaf water repellency of adaxial or abaxial surface is a main limiting factor which can affect the Zn uptake through spray application processes (Watanabe, T., I. Yamaguchi. 1991. Evaluation of wettability of plant leaf surfaces. Journal of Pesticide Science 16:491-498; Holder, C. D. 2007. Leaf water repellency of species in Guatemala and Colorado (USA) and its significance to forest hydrology studies. Journal of Hydrology 336:147-154). The permeability of the cuticle to water and to lypophilic organic molecules increases with mobility (distribution coefficients) and solubility (partition coefficients) of these compounds within the transport-limiting barrier of the cuticles. Ions being highly water soluble might have some hindrance in penetrating the lypophilic cuticle. This may be acting as a limiting factor in the case of chelated ZnSO4. But our custom made nanoscale ZnO, which is having less 1 hydrophilicity and being more dispersible in lypophilic substances compared to the ions, can penetrate through the leaf surface (Da Silva, L. C., M. A. Oliva., A. A. Azevedo., M. J. De Araujo. 2006. Response of resting plant species to pollution from an iron pelletisation factory. Water, Air and Soil Pollution 175:241-256) compared to ZnSO4. Also the mobility of the nanoparticles is known to be very high which

ensures the phloem transport and ensures the nutrient to reach all parts of the plant. The presence of nanoparticles both in the extracellular space and within some cells in the living plant Cucurbita pepo was reported (Gonzalez-Melendi, P., R. Fernandez Pacheco., M. J. Coronado., E. Corredor., P. S. Testillano., M. C. Risueno., C. Marquina., M. R. Ibarra., D. Rubiales., A. Perez-De-Luque. 2008. Nanoparticles as smart treatment-delivery systems in plants: assessment of different techniques of microscopy for their visualization in plant tissues. Annals of Botany 101:187-195). The increase in bioavailability of the nanoparticle may be because of its size and lower water solubility (which inhibits rapid falling-off compared to ionic supplements) compared to chelated $ZnSO_4$. The inherent small size and the associated large surface area of nanoscale ZnO fertilizer may increase the uptake as reported earlier. This enhanced uptake of Zn was seen in the EDAX analysis of the seeds also. All these factors may be responsible to give higher yields for nanoscale ZnO compared to chelated $ZnSO_4$.

[047] Based on application needs, the properties of nanoparticles can be fine tuned or engineered for its synthesis, size, shape, surface functionalisation and so on. The results presented in this invention used a specific kind of nanoparticle namely, ZnO, a method for its synthesis and applications thereof in agriculture.

We claim: **CLEAN COPY**

1. A method for preparing nanoscale monodisperse spherical ZnO particles of size 25 nm wherein the said method comprises
    a. preparing zinc oxalate from solutions of zinc acetate and oxalic acid or a combination thereof
    b. decomposing the zinc oxalate at a temperature range of 300-600 °C
    c. suspending the nanoparticles in water as a liquid suspension
2. The method as claimed in claim 1, wherein the size of the nanoparticles can be tuned till 100 nm.
3. The method as claimed in claim 1, wherein the nanoparticles has high surface area.
4. The method as claimed in claim 1, wherein the nanoparticle function as the vehicle for supplying Zn to crops
5. The method as claimed in claim 4, wherein the nanoparticles used in combination with N, P, K, copper, iron, manganese, boron and molybdenum in the form of mixed oxide or core-shell material.

Dated at Chennai this August 17, 2018

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ABSTRACT

METHOD FOR PREPARING NANOSCALE MONODISPERSE ZNO PARTICLES

A method for preparing zinc oxide nanoparticles by decomposing the zinc oxalate at a temperature range of 300-600 °C prepared from solutions of zinc acetate and oxalic acid or a combination thereof. The nanoparticles are suspended in water as a liquid suspension resulting in ~~a~~ spherical ZnO particles with uniform size distribution, high crystallinity, as well as favorable surface chemistry. The unique surface chemistry, size, and crystallinity of the ZnO NPs leads to enhanced uptake and Zn delivery in many applications.

METHOD FOR PREPARING NANOSCALE MONODISPERSE ZNO PARTICLES

Application Number: 3586/CHE/2011

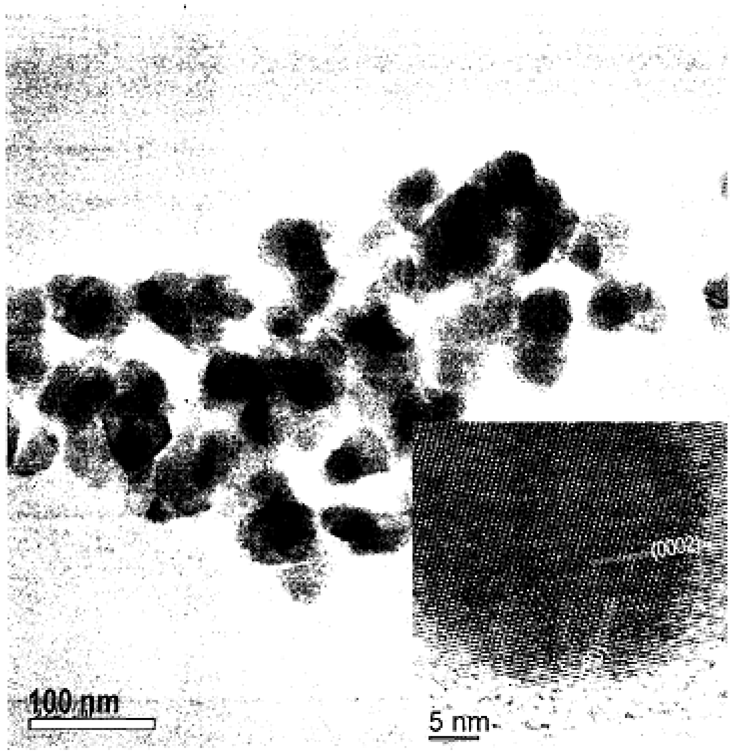

FIGURE 1

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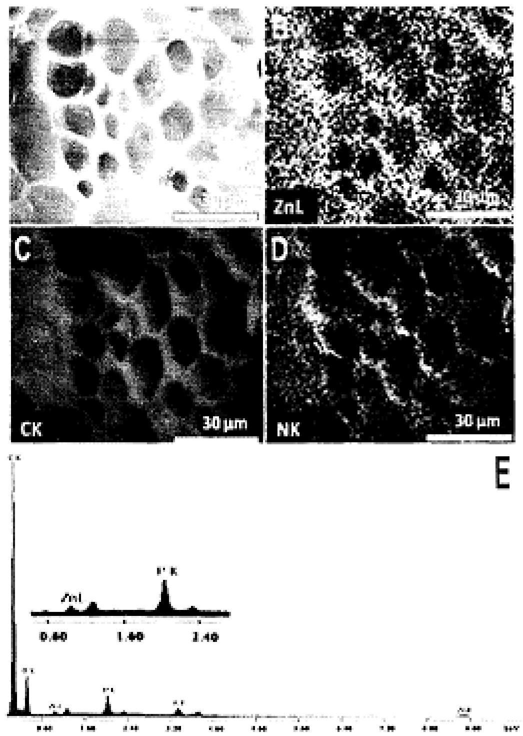

FIGURE 2

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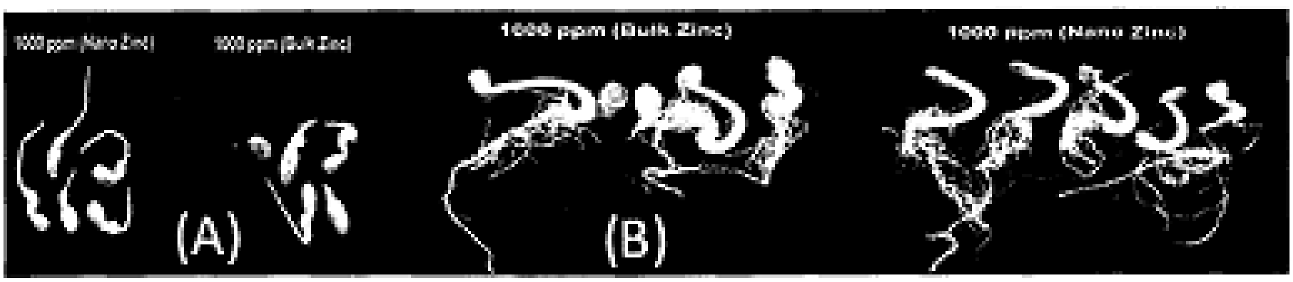

FIGURE 3

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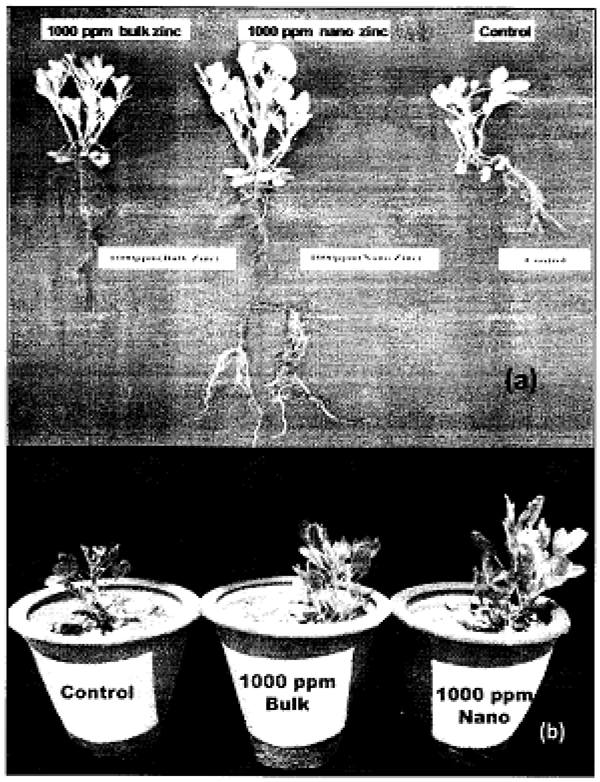

FIGURE 4

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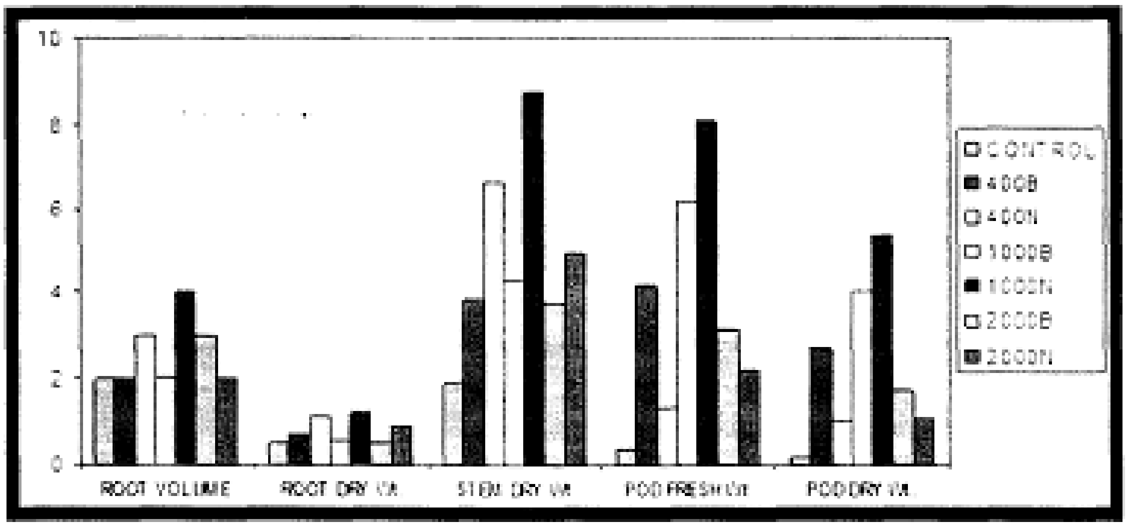

FIGURE 5

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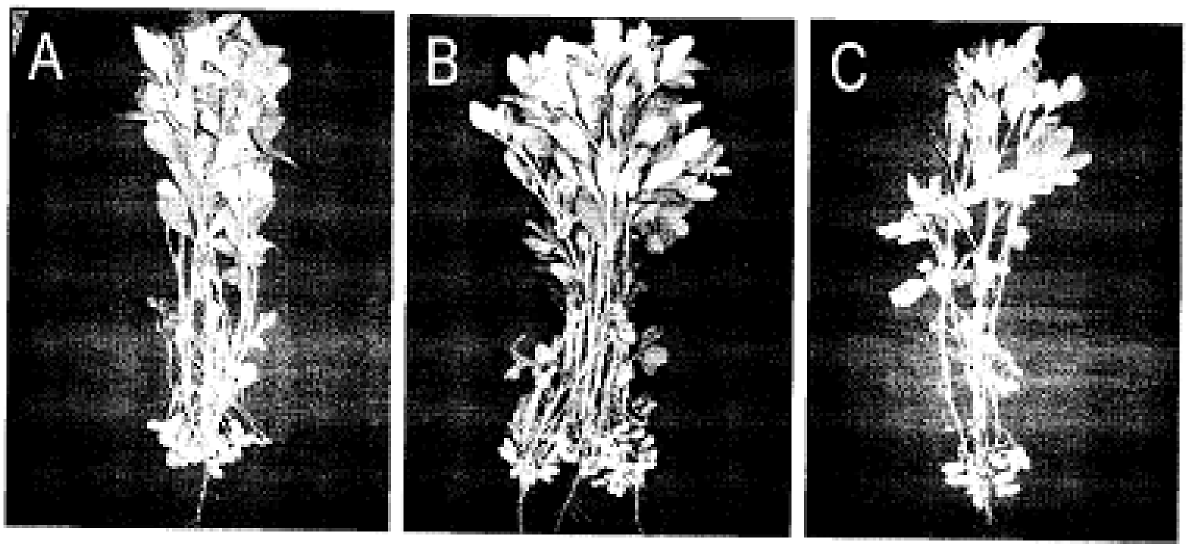

FIGURE 6

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METHOD FOR PREPARING NANOSCALE MONODISPERSE ZNO PARTICLES

Application Number: 3586/CHE/2011

Table 1: Effect of nanoscale ZnO and bulk $ZnSO_4$ on peanut mean germination and vigor

S.No.	Concentration (ppm)	Germination (%)		Shoot length (cm)		Root length (cm)		SVI	
		$ZnSO_4$	Nano ZnO	$ZnSO_4$	Nano ZnO	$ZnSO_4$	Nano ZnO	$ZnSO_4$	Nano ZnO
1.	400	84.01±0.94	90.33*±1.40	3.80±0.20	6.60*±0.18	5.84±0.12	11.52**±0.23	793.02**±6.83	1522.61**±12.32
2.	1000	90.32*±1.26	99.02**±1.41	4.32±0.15	8.71*±0.20	6.72*±0.19	11.81**±0.19	910.36**±8.56	1701.33**±9.89
3.	2000	88.75±1.29	96.04*±1.49	3.76±0.18	4.94±0.11	8.06*±0.21	9.42*±0.14	1195.72*±10.90	1321.74**±10.54
4.	Control	85.30		3.11		5.02		693.60	
	CD@5%	2.80		1.93		1.16		15.82	

Each value is the mean± SE of seven replicates.

Table 1

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METHOD FOR PREPARING NANOSCALE MONODISPERSE ZNO PARTICLES

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Table 2: Effect of nanoscale ZnO and bulk $ZnSO_4$ on peanut mean plant growth, flowering and leaf chlorophyll content

S.No.	Concentration (ppm)	Plant height (cm)		Initiation of flowering (days)		Chlorophyll content (mg/g fresh wt.)	
		$ZnSO_4$	Nano ZnO	$ZnSO_4$	Nano ZnO	$ZnSO_4$	Nano ZnO
1.	400	9.38*± 0.12	13.46**± 0.10	29.12±2.67	29.96±2.32	1.44*±0.01	1.66*± 0.02
2.	1000	12.42** ±0.09	15.40**± 0.02	29.01±1.94	27.24±1.65	1.74**±0.02	1.97**± 0.02
3.	2000	9.54*± 0.10	10.41**± 0.05	30.42±2.85	30.09±2.96	1.52*±0.01	1.76**± 0.01
4.	Control	8.22		29.00		1.39	
	CD@5%	0.16		NS		0.015	

Each value is the mean± SE of seven replicates.

Table 2

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METHOD FOR PREPARING NANOSCALE MONODISPERSE ZNO PARTICLES

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Table 3: Effect of nanoscale ZnO and bulk $ZnSO_4$ on mean root growth, shoot growth, dry weight and pod yield in peanut

S.No.	Concentration (ppm)	Root volume		Root dry wt (g)		Stem dry wt (g)		No.of filled pods/pl		Pod dry wt (g)	
		$ZnSO_4$	Nano ZnO	$ZnSO_4$	Nano ZnO	$ZnSO_4$	Nano ZnO	$ZnSO_4$	Nano ZnO	$ZnSO_4$	Nano ZnO
1.	400	2.20±0.10	3.20±0.08	0.72* ±0.05	1.21** ±0.02	3.84* ±0.21	6.64*± 0.26	1.93± 0.01	1.96±0.07	2.70* ±0.09	3.04* ±0.16
2.	1000	2.10±0.09	4.22±0.10	0.54± 0.01	1.20** ±0.06	4.29* ±0.15	8.72** ±0.18	5.96* *±0.04	6.59±** 0.01	3.97* ±0.07	5.39** ±0.11
3.	2000	3.21±0.16	2.16± 0.06	0.47± 0.02	0.92* ±0.03	3.75* ±0.20	4.96 *±0.22	3.05* ±0.03	2.04±0.02	1.70± 0.02	1.09±0.04
4.	Control	2.10		0.47		1.91		2.00		1.18	
	CD@5 %	NS		0.07		0.01		0.08		0.60	

Each value is the mean± SE of seven replicates.

Table 3

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METHOD FOR PREPARING NANOSCALE MONODISPERSE ZNO PARTICLES

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Table 4: Response of peanut to application of nano ZnO

S.No	Treatment	Plant height in (cm)	No. of branches per plant	No. of pods per plant	No. of filled pods per plant	No. of Ill filled pods per plant
1.	T1 = NPK (Control)	36.50±1.20	3.85±0.42	9.20±1.89	8.20±1.80	1.00±0.04
2.	T2 = NPK + $ZnSO_4$(Chelated)@30g/15l	37.10±1.98	3.85±0.78	10.10±2.42	9.10±2.01	1.00±0.02
3.	T3 = NPK + ZnO (Nano)@2g/15l	43.80*±2.10	4.57±0.65	16.80*±2.01	15.00*±1.98	1.80±0.05
	CD@5%	4.47	NS	3.76	2.99	0.92

Each value is the mean± SE of seven replicates.

Table 4

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Table: 5 Effect of nano ZnO on yield and yield attributes of peanut

(Rabi season 2008-09)

S.No	Treatment	Pod yield (kg/ha)	100 pod weight (g)	100 kernel weight (g)	Shelling percentage
1.	T1 = NPK (Control)	2391.56±38.40	77.27±1.52	31.50±2.08	63.81±2.26
2.	T2 = NPK + $ZnSO_4$(Chelated)@30g/15l	2410.82±72.86	74.82±0.58	30.92±1.96	64.62±2.17
3.	T3 = NPK + ZnO (Nano)@2g/15l	3121.54**±115..23	83.90**±0.46	36.25*±2.14	67.50**±1.45
	CD@5%	199.92	2.89	2.52	2.68

Each value is the mean± SE of seven replicates.

Table 5

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METHOD FOR PREPARING NANOSCALE MONODISPERSE ZNO PARTICLES

Application Number: 3586/CHE/2011

Table: 6 Effect of nano ZnO on yield and yield attributes of peanut

(Rabi season 2009-10)

S.No.	Treatment	Pod yield (kg/ha)	100 pod weight (g)	100 kernel weight (g)	Shelling percentage
1.	T1 = NPK (Control)	2711.78±25.34	83.26±1.11	31.60±0.56	60.22±0.21
2.	T2 = NPK + $ZnSO_4$(Chelated)@30g/15l	2978.42*±39.71	112.14*±1.78	37.82*±0.22	66.97*±0..72
3.	T3 = NPK + ZnO (Nano)@2g/15l	3763.65**±56.09	117.80**±2.43	47.91**±0.34	69.30**±0.65
	CD@5%	90.78	3.49	0.98	1.03

Each value is the mean± SE of seven replicates.

Table 6

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METHOD FOR PREPARING NANOSCALE MONODISPERSE ZNO PARTICLES

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Table: 7 Effect of nanoscale ZnO on uptake of Zn by leaf and kernel of peanut

S.No.	Treatment	Zinc content (ppm) 2008-'09 (Rabi season)		Zinc content (ppm) 2009-'10(Rabi season)	
		Leaf(post harvest)	Kernel	Leaf(post harvest)	Kernel
1.	T1 = NPK (Control)	22.31±1.08	21.84±0.67	22.81±1.31	20.46±0.56
2.	T2 = NPK + $ZnSO_4$(Chelated)@30g/15l	31.46*±1.05	28.32*±0.84	32.36*±0.93	29.21*±0.76
3.	T3 = NPK + ZnO (Nano)@2g/15l	44.80**±1.08	40.20**±0.31	41.83**±1.06	39.90**±0.89
	CD@5%	1.50	1.36	1.46	1.35

Each value is the mean± SE of seven replicates.

*Significant at p (level of probability) is less than 0.05
**Highly significant at p (level of probability) is less than 0.05

Table 7

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