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NOVEL PROSPECTIVE FOR CHITOSAN BASED NANO-MATERIALS IN PRECISION AGRICULTURE - A REVIEW

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ABSTRACT
Recent expansions in nanotechnology have led to the creation of various chitosan based nano-materials. These chitosan based nano-materials will have prominent task in transforming agriculture. Chitosan is a bio-compatible, biodegradable, non-toxic material and could be an alternative to synthetic agrochemicals applied in agriculture. The literature pertaining to chitosan use in agriculture reveals that chitosan nanomaterial's will have imperative role in the controlled release of agrochemicals for protection against pests and pathogens, delivery of genetic material, soil fortification, drought tolerance and plant growth. This review recaps the role of chitosan nano-materials in agriculture.

INTRODUCTION

Presently, agricultural sector facing the various hurdles with change in the climate, urbanization, diminishing agricultural land, death of natural resources and meet the growing demands of the food. Hence there is more emphasis on sustainable and precision agricultural practices. Recent developments in the science and technology such as nano-technology boon to the agriculture. The concepts of nanotechnology seeded in 1959 by Richard Feynman. He described the possibility of synthesis via manipulation of atoms directly. The term "nano-technology" was first used by Norio Taniguchi in 1974. One nanometre (nm) is one billionth, or 10^{-9}, of a meter. In nanotechnology, basically two main approaches are used- bottom-up and top-down. In the "bottom-up" approach, materials and devices are built from molecular components which self-assemble chemically by principles of molecular recognition. In the "top-down" approach, nano-objects are constructed from larger entities without atomic-level control. Nanomaterials are materials with a particle size less than 100 nm in at least one dimension.

Chitosan is most abundant carbohydrate biopolymer in the world. Chitosan is a linear polysaccharide made up of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit), produced commercially by deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans (such as crabs and shrimp) and cell walls of fungi (figure 1). Chitosan have special unique properties such as high surface area, adsorption properties ,viscosity, soluble in various media, positive charge, basic polysaccharide, non-antigenicity, ideal candidate for bio-fabrication, polyelectrolyte behaviour, ability to form films, metal chelation etc. Chitosan and its derivatives are attracted considerable interest among the agricultural researchers due to their biocompatibility, antimicrobial nature, environmentally safer (nontoxic) and biodegradability biodegradable and friendly to environment. In the field of agriculture it can be used as a bio-pesticide, controlled release of the fertilizer or growth regulators, packing materials, plant growth and development and also in efficient abiotic stress management.

The poly-cationic nature of chitosan is importance in antimicrobial activity. A high positive charge present in the chitosan makes it suitable candidate for bio-control agent in agriculture. Chitosan elicits defence responses in plant to resist insects and various bacterial and fungal pathogens (Linden *et al.*, 2005). The degree of deacetylation (DD), pH, Ionic strength, molecular weight and many factors as so play parallel role in antimicrobial activity in addition to positive charge density (Kong, *et al.*, 2010). Tremendous literatures evidenced shows chitosan can also efficiently enhances photosynthesis, promotes plant growth, increases nutrient uptake, germination and sprouting. Overall it boosts plant growth and its defense against biotic and abiotic stress. Chitosan has ability to bind with negative charged bio-molecules, thus can act as good carrier for nucleic acid and applied for delivery of genetic materials in biological system. Thus, in agriculture, chitosan nanomaterials could be used for plant defense and plant growth(Hadwiger, 2013, Rodriguez *et al.*, 2011, Menget *al.*, 2010, Ghormade *et al.*, 2011, Khot *et al.*, 2012, Saharan *et* *al*., 2013, Saharan *et al*., 2014, Raut *et al*., 2014). Chitosan nanoparticles have been developed based on the cross linking of chitosan with tripolyphosphate (TPP) by various groups. This physical cross-linking process not only avoids the use of chemical cross-linking agents and emulsifying agents which are often toxic to organisms, but also prevents the possibility of damage to biological agents. Therefore, nano-chitosan has more biological activity as compared to its bulk form. Thus chitosan based nanomaterial has showed a wide range of applications in precision agriculture and explain in following headings

Applications of chitosan based nanomaterials in agriculture

role of chitosan in biotic stress management

Due to global warming, micro-organisms are frequently changing their behaviour to endure in the changing environment. Therefore, measurements which are in place to control plant disease are inadequate. Antifungal compounds which are in use, losing their activity rapidly. In addition, synthetic nature of fungicides also contaminates environment and deposited into food chain. Further, uncontrolled use of available fungicides have raised serious problem of resistance development. There is urgent need to emphasize on evaluation of novel antimicrobial compounds which are natural and having multiple targets sites on disease causing micro-organisms so that resistance development can be delayed. Blending of nano-science with biogenic substances has set off a novel field of research known as nano-biotechnology. In future, nano-biotechnological approaches would be the alternative to existing technologies for management of plant diseases (Saharan *et al*., 2013).Plant pathogens considered efficiently important agricultural micro-organisms around the world. They induce decay on a various agricultural crops during the growing season and postharvest. Chitosan induces as well as enhances the defence responses in plants when pathogen invades. The responses such as reactive oxygen species, membrane depolarization, biosynthesis of ABA, salicylic acid, jasmonic acid, hypersensitive responses, production of phytoalexins, callose formation, programmed cell death (Iriti and Faoro, 2008, Jayalakshmi *et al*., 2010) and expression of defence related genes etc. (Hadrami *et al*., 2010).

The modification of chitosan nanoparticles loaded with the metal ions such as chitosan - Ag^{+} or Cu^{2+}/Zn^{2+} complexes exhibited high antimicrobial activity (Du *et al*., 2009). The plant protection activities of chitosan compounds have been well renowned in many plant systems. Up to now, there is a sufficient amount of verification indicating that after chitosan application, plants can attain enhanced tolerance to a wide variety of pathogenic microorganisms (Badawy and Rabea, 2011, Khot *et al*., 2012, Shukla *et al*., 2013, Hadrami *et al*., 2011).

The antimicrobial activity of modified chitosans against plant pathogenic bacteria of crown gall disease *Agrobacterium tumefaciens* and soft mould disease *Erwiniacarotovora* and fungi of early blight disease *Alternariaalternata*, root rot disease *Fusarium oxysporum*, and damping off disease *Pythium debaryanum* (reported by Rabea and Steurbaut, 2010.) Antiviral activity of chitosan also reported with potato virus X, tobacco mosaic virus, peanut mosaic virus and cucumber mosaic viruses. Probably chitosan inhibit the systemic propagation of the virus in the plant due enhancing the hypersensitive reactions avoid cell- to cell movement of virus (kulikov*et al*., 2006). Modified chitosan molecules shows enhanced bioactivity against some plant pathogenic bacteria and fungi. The effect of chitosan-copper nanoparticles on antibacterial and antifungal activity of several microorganisms *(Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Salmonella choleraesuis, Alternaria alternata, Macrophomina phaseolina, Rhizoctonia solani* and *Candida albicans)* was studied (Usman *et al*., 2013, Saharan *et al*., 2013, Qi *et al*., 2004).

Plant growth and seed germination

Enhancing of growth in the plants have been reported in range crops. Significant growth and development reported after application of chitosan in the crops such as *Raphanus sativa, Brassica oleracea, Gerbera* and *Dendrobium* orchids etc. (Sharp, 2013). Elexa, a chitosan formulation for treatment of pearl millet seeds for growth promoting effect under greenhouse conditions and found that chitosan induced overall growth of plant (Sharathchandra*et al*., 2004). Seed priming with different concentrations of chitosan solutions on the growth and physiological changes of two maize (*Zea mays* L.) inbred lines were studied and concluded that chitosan enhanced germination index, reduced the mean germination time and increased shoot height, root length, and shoot and root dry weights in both maize lines. Thus, it suggests that seed priming with chitosan may increase the germination speed of maize seeds and also beneficial for seedling growth (Guan *et al*., 2009).

Bulk chitosan stimulate germination and growth of ajowan and alleviate the inhibitory effect of salt stress on the plant growth (Mahdavi and Rahimi, 2013). Chitosan coated seeds of various crops like peanut, soybean, maize, wheat cabbage, rape etc. enhance the germination, growth and yield (Zhou *et al*., 2002, Shao *et al*., 2005, reddy *et al*., 1999, Wang *et al*., 2012). The growth of plants and their quality depends mainly on quantity of fertilizer and water. So soil fortification is also important to improve the utilization of water resources and fertilizer nutrients. Chitosan-coated nitrogen (N), phosphorus (P) and potassium (K) were tested and found effective in providing nutrition to plant more precisely compared to sole NPK application (Wu and Liu, 2008, Corradini *et al*., 2010).

Role of chitosan in abiotic stress management

Chitosan has repeatedly been shown to extremely avoid the

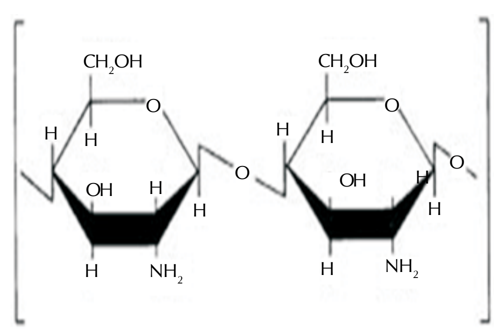

Figure 1: Structure of chitosan

abiotic stress due its antioxidant activity makes it effectively scavengers of hydroxylated radicles, hydrogen peroxidase etc. (Chen *et al.*,2009). Chitosan reduces the water use by stomatal closure and reducing the transpiration have been reported in the pepper plants about 26-43%. Rice plants treated with chitosan shows higher yield than control (Bittelli *et al.,* 2001). Studied the effect of chitosan applicationto maintain growth and yield potential of rice plants encountering drought stress by applying chitosan by soaking the seeds before planting followed by foliar spray.Effects of chitosan and chitosan oligomer solutions on biophysical characteristics, growth, development and drought resistance of coffee, strawberry etc. *have been studied (Dzung et al.,* 2011*,* Caulet *et al.*, 2014*)*. Thaler and Bostock, (2004) studied the interactions between abscisic acid mediated responses and plant resistance to pathogens and insects. Paulin *et al.*, 2011 studied the effect of chiotosan application in maize to neutralize the effects of abiotic stress. Chitosan might be an effective antitranspirant to conserve water use in agriculture. Chitosan influence the jasmonic acid synthesis which plays a key role in the regulation of water use by plants as abscisic acid. Increased levels of ABA results in stomata closure and reduced transpiration (Bittelli *et al.*, 2001, Iriti *et al.*, 2009).

Control releasing of formulations

Controlling release of formulations (CRFs) are promising in improving the efficiency of pesticides, growth regulators or fertilizers and minimizing the spreading of hazardous residues in environment. CRFs are advantageous for maintaining an optimum concentration of substances over a period of time, providing more effective biological effect (Maqueda *et al.*, 2008). nano-based smart delivery systems can be used for controlled release of agrochemicals for protection against pests and pathogens, to intellect the presence of plant viruses, soil nutrient content and nanoencapsulated slow release of fertilizers without waste by leaching (Ingale and Chaudhari, 2013, Naderi and Shahraki, 2013, DeRosa *et al.*, 2010).Natural polymers such as pectin, starch, cellulose and chitosan are desirables for carries of substance. Among the natural polymers chitosan shows greater potentiality in development of economical; and versatile materials of CRFs. Chitosan possess a high encapsulation capacity to pesticides or GR or fertilizers. Recently reported Gibberellins conjugated with chitosan promising CRFs which play impotent role in seed germination, flower development and epidermal elongation in the plants (Liu *et al.*, 2013).

Biosensors and Delivery of Genetic Material

Nanotechnology has important role in various areas of basic research to support the sustainable agriculture for improving crop productivity. Increasing concentration of heavy metals, herbicides, pesticides, fertilizers etc. in agricultural land is harmful and this ultimately causes decrease in production and productivity. Thus to revolutionize the agricultural system biosensors are of a great value. Nano-biosensor opens up new opportunities for agriculture field. Nano-biosensor is a compact analytical device incorporating a biologically derived sensitized element linked to a physico-chemical transducer (Turner *et al.*, 2000). Nano-biosensors can be used for sensing a wide range of fertilizers, herbicide, pesticide, insecticide, pathogens, moisture, soil quality like pH humidity and various soil microorganisms. Nano-biosensor in agriculture can be used as (Rai *et al.*, 2012)

Diagnostic tool for monitoring soil condition and disease estimation.

Detection of microbial contamination.

An agent to advance sustainable agriculture.

As a valuable tool for detection of genetic material and nutrients and plant hormones.

Liu *et al.*, 2008 have reviewed the use of nano-biosensors for detection of organophosphate pesticide. Various Colorimetric methods, mass spectrometric methods and chromatographic methods can be used for detection of pesticide residues in various agricultural products. Analysis can be done by recording the changes in color, fluorescence or electrical potential (Li *et al.*, 2013). Enzyme based biosensors have been developed like tyrosinase biosensor for the detection of catechol, liposome-based nano-biosensors for detection of organophosphorus pesticides dichlorvos and paraoxon, nano biosensors for organochlorines, organophosphates, and carbamates residue detection (Wang *et al.,* 2012, Dyk and Pletschke 2011). Research on nano-biosensors for their application in agriculture would be greatly helpful for speedy disease identification and disease management and for better productivity and production in agriculture.

Success of delivery of genetic material is mainly limited due to the lack of effective vector systems. Chitosan nanoparticles can be used as non-viral vector offering several advantages, such as biocompatibility, biodegradability and low toxicity with high cationic potential (Kim *et al.*, 2007). Nanoparticle gene carriers have considerable advantages compared with traditional carriers like applicable to both monocotyledons and dicotyledonous plants, overcome transgene silencing *via* controlling the copies of DNA combined to nanoparticles, enhance transformation efficiency and multigene transformation can be achieved. Nanofibre arrays can also be used for delivery of genetic material to cells quickly and efficiently (Nair *et al.*, 2005). Therefore chitosan nanoparticles can be used as a gene carrier in plant genetic engineering.

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REFERENCES

Badawy, M. E. I. and Rabea, E. I. 2011. A biopolymer chitosan and its derivatives as promising antimicrobial agents against plant pathogens and their applications in crop protection.*Int. J. Carbohydr. Chem.* **1:** 1-29.

Bittelli, M., Flury, M., Campbell, G. S. and Nichols, E.J. 2001. Reduction of transpiration through foliar application of chitosan. *Agric. For Meteorol.* **107:** 167-175.

Boonlertnirun, S., Suvannasara, R. and Boonlertnirun, K. 2013. Effects of chitosan application before being subjected to drought on physiological changes and yield potential of rice (*Oryza sativa* L.). *J. Appl. Sci. Res.* **9(12):** 6140-6145.

Caulet, R. P., Gradinariu, G., Iurea, D. and Morariu, A. 2014. Influence of furostanolglycosides treatments on strawberry (Fragaria×ananassa Duch.) growth and photosynthetic characteristics under drought condition. *Sci. Hortic.* **169:** 179-188.

Chenguang, L., Yulong, T., Chengsheng, L., Xiguang, C. and Lejun, Y. 2007. Preparations, characterizations and applications of chitosan-based nanoparticles.*J. Ocean Univ. China.* **6:** 237-243.

Corradini, E., de Moura, M. R. and Mattoso, L. H. C. 2010. A preliminary study of the incorparation of NPK fertilizer into chitosan nanoparticles. *Express Polym Lett.* **4(8):** 509-515.

DeRosa, M. C., Monreal, C., Schnitzer, M., Walsh, R. and Sultan, Y. 2010. Nanotechnology in fertilizers. *Nat. Nanotechnol.* **5:** 91-94.

Dzung, N. A., Khanh, V. T. P. and Dzung, T. T. 2011. Research on impact of chitosan oligomers on biophysical characteristics, growth, development and drought resistance of coffee. *Carbohydr. Polym.* **84:** 751-755.

Guan, Y. J., Hu, J., Wang, X. J. and Shao, C. X. 2009. Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress. *J Zhejiang. Univ. Sci. B.* **10:** 427-433.

Ghormade, V., Deshpande, M. V. and Paknikar, K. M. 2011. Perspectives for nanobiotechnology enabled protection and nutrition of plants. *Biotechnol. Adv.* **29:** 792-803.

Hadrami, A. E. I., Adam, L. R., Hadrami, I. E. and Daayf, F. 2011. Chitosan in plant protection.*Mar Drugs.* **8:** 968-987.

Hadwiger, L.A. 2013. Multiple effects of chitosan on plant systems: Solid science or hype. *Plant Sci.* **208:** 42-49.

Ingale, A. G. and Chaudhari, A. N. 2013. Biogenic synthesis of nanoparticles and potential applications: An eco-friendly approach. *J NanomedNanotechol.*4: 165. doi:10.4172/2157-7439.1000165.

Iriti, M. and Faoro, F. 2008. Abscisic acid is involved in chitosan-induced resistance to tobacco necrosis virus (TNV). *Plant Physiol Biochem.* **46:** 1106-1111.

Iriti, M., Picchi, V., Rossoni, M., Gomarasca, S., Ludwig, N., Gargano, M. and Faoro, F. 2009. Chitosan antitranspirant activity is due to abscisic acid-dependent stomatal closure. *Environ. Exp. Bot.* **66:** 493-500.

Jayalakshmi, P., Suvarnalathadevi, P., Prasanna, N. D., Revathi, G. and Shaheen , S. K. 2010. Morphological and physiological changesof Groundnut plants by foliar application with salicylic acid.*The Bioscan.* **5(2):** 193-195.

Khot, L. R., Sankaran, S., Maja, J. M., Ehsani, R. and Schuster, E. W. 2012. Applications of nanomaterials in agricultural production and crop protection: A review. *Crop Prot.* **35:** 64-70.

Kim, T. H., Jiang, H. L., Jere, D., Parka, I.K., Cho, M.H. and Nah, J. W. 2007. Chemical modification of chitosan as a gene carrier *in vitro* and *in vivo*. *Prog. Polym. Sci.* **32:** 726-753.

Lan, Wu, L. and Liu, M. 2008. Preparation and properties of chitosan-coated NPK compound fertilizer with controlled-release and water-retention. *Carbohydr Polym.* **72:** 240-247.

Liu, S., Yuan, L., Yue, X., Zheng, Z. and Tang, Z. 2008. Recent advances in nanosensors for organophosphate pesticide detection. *Adv Powder Technol.* **19(5):** 419-441.

Li, W., Sun, M. and Li, M. 2013. A survey of determination for organophosphorus pesticide residue in agricultural products. *Adv. J. Food Sci. Technol.* **5(4):** 381-386.

Linden, J. C., Stoner, R. R., Knutson, K. W., Gardner, H. and Cecilie, A. 2000. Organic disease control elicitors. *Agro food industry Hi-Tech.* **11(5):** 32-4.

Linden, J. C. and Stoner, R. R. 2005. Proprietary elicitor affects seed germination and delays fruit senescence. *J. Food Agric. Environ.* **3:** 184-189.

Lizarraga-Paullin, E. G., Torres-Pacheo, Moreno-Martinez, E. and Miranda-Castro, S. P. 2011. Chitosan applicaton in maize (*Zea mays*) to counteract the effects of abiotic stress at seedling level.*Afr. J. Biotechnol.* **10(34):** 6439-6446.

Mahdavi, B. and Rahimi, A. 2013. Seed priming with chitosan improves the germination and growth performance of ajowan (*Carumcopticum*) under salt stress. *Eurasia J. Biosci.* **7:** 69-76.

Meng, X., Yang, L., Kennedy, J. F. and Tian, S. 2010. Effects of chitosan and oligochitosan on growth of two fungal pathogens and physiological properties in pear fruit. *Carbohydr Polym.* **81:** 70-75.

Naderi, M. R. and Danesh-Shahraki, A. 2013. Nanofertilizers and their roles in sustainable agriculture. *Intl. J. Agri. Crop. Sci.* **5(19)**: 2229-2232.

Nair, R., Varghese, S. H., Nair, B. G., Maekawa, T., Yoshida, Y. and Kumar, D. S. 2010. Nanoparticulate material delivery to plants, *Plant Sci.* **179:** 154-163.

Qi, L., Xu, Z., Jiang, X., Hu, C. and Zou, X. 2004. Preparation and antibacterial activity of chitosan nanoparticles. *Carbohydr. Res.* **15: 339(16):** 2693-2700.

Rabea, E. I. and Steurbaut, W. 2010. Chemically modified chitosans as antimicrobial agents against some plant pathogenic bacteria and fungi. *Plant Protect. Sci.* **46:** 149-158.

Rai, V., Acharya, S. and Dey, N. 2012. Implications of nanobiosensors in agriculture. *J. Biomater Nanobiotechnol.* **3:** 315-324.

Raut, S. A., Borkar, S. G. and Nagrale, D. T. 2014. Effect of disease (alternaria leaf blight) resistance elicitors on growth parameters of tomato plant. *The Bioscan.* **9(3):** 1157-1159.

Reddy, M. V., Arul, J., Angers, P. and Couture, L.1999. Chitosan treatment of wheat seeds induces resistance to *Fusarium graminearun*and improves seed quality. *J. Agric. Food Chem.* **47:** 1208-1216.

Rodriguez, A. B. F., Costales, D., Cabrera, J. C. and Téllez, M. A. M. 2011. Chitosan physic-chemical properties modulate defense responses and resistance in tobacco plants against the oomycete *Phytophthoranicotianae*. *Pest Biochem Physiol.* **100:** 221-228.

Saharan, V., Mehrotra, A., Khatik, R., Rawal, P., Sharma, S. S. and Pal, A. 2013. Synthesis of chitosan based nanoparticles and their *in vitro* evaluation against phytopathogenic fungi. *Int. J. Biol. Mac.* **62:** 677-683.

Saharan, V., Khatik, R., Choudhary, M. K., Mehrotra, A., Jakhar, S., Raliya, R., Joshi, A., Nallamuthu, I. and Pal, A. 2014. Nano-materials for plant protection with special reference to Nano-chitosan, In: proceedings of 4th Annual International conference on Advances in Biotechnology, *GSTF*, Dubai, pp. 23-25.

Shao, C. X., Hu, J., Song, W. J. and Hu, W. M. 2005. Effects of seed priming with chitosan solutions of different acidity on seed germination and physiological characteristics of maize seedling. *J. zhejian guniversity (Agriculture and life sciences).* **1:** 705-708.

Shukla, S. K., Mishra, A. K., Arotiba, O. A. and Mamba, B. B. 2013. Chitosan-based nanomaterials: A state-of-the-art review. *Int. J. Biol. Mac.*, doi:10.1016/j.ijbiomac.2013.04.043.

Smiley, R., Cook, R. J. and Pauliz, T. 2002. Seed treatment for sample cereal grains. *Oregon state university, EM 8797.*

Thaler, J. S. and Bostock, R. M. 2004. Interactions between abscisic acid mediated responses and plant resistance to pathogens and insects, *Ecology*. **85:** 45-58.

Turner, A. P. 2000. Biosensors-sense and sensitivity. *Science*. **290:** 1315-1317. doi:10.1126/science.290.5495.1315.

Usman, M. S., Zowalaty, M. E. E., Shameli, K., Zainuddin, N., Salama, M. and Ibrahim, N. A. 2013. Synthesis, characterization, and antimicrobial properties of copper nanoparticles. *Int. J. Nanomedicine.* **8:** 4467-4479.

Wang, L., Li, B., Chen, X., Shi, Y., Zhou, Q., Qiu, H., Ibrahim, M., Xie, G. L. and Sun, G. C. 2012. Effect of chitosan on seed germation, seedling growth and the clubroot control in Chinese cabbage.*J Food Agric Environ*, **10 (3&4):** 673-675.

Wang, S., Tana, Y., Zhaoa, D. and Liu, G. 2008. Amperometricty rosinase biosensor based on Fe3O4 nanoparticles-chitosan nanocomposites, *Biosens. Bioelectron*. **23:** 1781-1787.

Xu, J., Zhao, X., Han, X. and Du, Y. 2007.Antifungal activity of oligochitosan against *Phytophthoracapsici* and other plant pathogenic fungi *in vitro*. *Pest Biochem Physiol*. **87:** 220-228.

Zeng, D. and Luo, X. 2012. Physiological Effects of Chitosan Coating on Wheat Growth and Activities of Protective Enzyme with Drought Tolerance. *Open J. Soil Sci*. **2:** 282-288.

Zeng, D., Luo, X. and Tu, R. 2012. Application of bioactive coatings based on chitosan for soybean seed protection. *Int. J. Carbohydr. Chem*. pp. 1-5.

Zhou, Y. G., Yang, Y. D., Qi, Y. G., Zhang, Z. M., Wang, X. J. and Hu, X. J. 2002. Effects of chitosan on some physiological activity in germinating seed of peanut. *J. Peanut Science*. **31(1):** 22-25.