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Development of next-gen nano-bio formulation of *Trichoderma asperellum* against soil borne pathogens

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Abstract
In the present study, chitosan nanoparticles were synthesized from *Trichoderma asperellum* and one commercial product of chitosan (Sigma Aldrich) was taken for comparison. Formation of chitosan nanoparticles (NPs) were confirmed by UV-VIS spectroscopy study with absorption peaks at the range of 310.02 to 342.00 nm. FTIR study showed that synthesized chitosan NP has all the required functional groups like OH, N-H, C-H, C=O, C-O, C-N and P=O. Study on surface properties of NPs by using zetasizer resulted that chitosan NPs synthesized from *T. asperellum* and commercial product were found to be positively charged and were stable in nature with zeta potential of 9.78 and 17.6 mV respectively. Electron microscopy study showed that the shape of NP as nearly spherical. DLS analysis showed the average size of the chitosan NPs synthesized from *T. asperellum* and commercial products as 89.03 and 300.10 nm respectively.

Keywords: Formulation, *Trichoderma asperellum*, chitosan, shelf life, nanoparticles

Introduction
Fusarium oxysporum Schlecht and *Sclerotium rolfsii* Sacc are soil borne plant pathogenic fungi which can cause diseases to various field and horticultural crops with significant yield loss. Fusarium species cause damage in crops *viz.*, potato, pea, bean, wheat, corn and rice resulted in yield losses of about 30- 70 percent (Saremi *et al.*, 2011) [17]. Fusarium wilt disease caused by *F. oxysporum* remains as a major threat to vegetable production worldwide. Soil borne nature of the pathogen coupled with its ability to survive in the form of chlamydospores for about 3 decades in infested plant debris or in the roots of alternate hosts adds to its highly potential nature of infecting crop plants (Saikia, 2004) [16].
S. rolfsii Sacc (Teleomorph: *Athelia rolfsii*), an omnivorous, soil borne fungal pathogen, causes disease on a wide range of crops. It occurs worldwide but is most important in tropical and subtropical regions. It produces abundant white mycelium on infected plants. The wide host range, prolific growth, and ability to produce persistent sclerotia (0.5 to 1.5 mm in diameter) contribute to the large economic losses (1-60%) in different crops (Kwon *et al.*, 2002) [8].
Various studies relating to the management of these pathogens have been carried out and reports suggests that a single management practice has not been very effective in managing these diseases because of the soil borne nature as well as ability to survive for years. Besides this, the detrimental effect of chemical pesticides brings the urgent need of an effective biological control measure, which is an important, effective, eco-friendly and economical component of Integrated Pest Management (IPM) in almost all important crops for development of sustainable cropping systems.
Trichoderma is an antagonistic biological control agent of potential soil borne pathogens. During last decade, species of *Trichoderma* have emerged as one of the powerful bio protectants for management of wide variety of plant diseases. This is truer in the context of the fact that there is considerable public pressure and pressure from environmental Scientists to reduce emphasis on chemical protectants and use bio protectants. The genus *Trichoderma* by virtue of its broad spectrum action against a number of plant diseases caused by fungi, bacteria, viruses and even nematodes has occupied the top position among the bio protectants developed for plant disease management.

Trichoderma based bioformulation has been developed by different scientists throughout the world with effective field results. Chitosan, a cationic polysaccharide (partly de-acetylated form of chitin) can elicits natural innate defense responses within the plant system to resist insects, pathogens and soil borne diseases when applied to foliage or the soil. *Trichoderm*a sp. can be stimulated for production of Chitinase by addition of nano chitosan in its bio-formulation (Mauch *et al.*, 1988) [9]. Chitosan also increases photosynthesis, promotes and enhances plant growth, stimulate nutrient uptake, increase germination and sprouting and boost plant vigour. Besides of its ability as a barrier to the growth of plant pathogenic fungi, chitosan is reported to act as a potential elicitor for various defense responses in plants. The efficiency of chitosan based nano-formulations of *Trichoderma* depends on the use of efficient strains, its formulation and the environment in which it is used.

Material and Methods

Culture of bio-agents: The pure fungus culture of *Trichoderma asperellum* (ITCC No. 8886.12) were collected from the culture bank maintained under Peak Chemical Industries Limited (PCIL) funded project of Department of Plant Pathology, AAU, Jorhat.

Maintenance of culture

Pure culture of all the collected fungus was maintained on PDA medium by periodic transfer in fresh medium. A loopful of inoculum from cultured tubes were transferred to PDA plates (9 cm diameter), incubated in the BOD incubator (Orbitek) for 7 days at 28±1 °C and maintained as pure culture. After complete sporulation, for subsequent studies the fungus was sub cultured and stored in refrigerator at 4 °C.

Sources of chitosan

Trichoderma asperellum have been used for isolation of chitosan and one commercial source of chitosan (Sigma Aldrich) (Plate 1).

Plate 1: *Trichoderma asperellum* and Chemical product of Chitosan

Isolation of chitosan from fungus cell wall

Isolation of chitosan from fungal cell wall was done by following the standard method of Aghdam, 2010 [2] (Plate 2). In that method first the biomass of different fungus was harvested on a screen, washed it with sterile water and kept in refrigerator for freezing. Than that biomass was dried in ventilated cabinet dryer at 40°C and made the biomass as a powder with coffee grinder (USHA, MG-2853) Weighted the biomass and divide it into 2 g portions. Kept each portion in a 100 ml conical flash, added sodium hydroxide solution (NaOH 2%, 60 ml) into it and then sterilized it in the autoclave at 120 °C for 2 minutes. Centrifuged the mixture, get the solid and washed it several times with water to get natural pH (This solid was named as Alkali insoluble material, AIM, which was our cell wall). Kept that AIM at freeze and then it was freeze dried. Took 0.25 g of the AIM for H2SO4 treatment. Treated AIM samples were kept in the incubator at 120 °C for 20 minutes. After incubation tube solutions were transferred on filter rapidly for keeping the temperature in about 90 °C. Then the liquid A was separated for one have on ice which was washed repeatedly to neutral pH. Filter papers were kept in oven at 50 °C for drying. Centrifuged the liquid A then kept the aqueous phase of liquid A after that washed the solid phase of liquid A to neutral pH, washed it with acetone for extraction improvement as a pure polysaccharide and then dried the solid.

Plate 2 (a-i): Different steps involved in the process of preparation of chitosan
a. Pure culture of the fungus, b. Mass culture of the fungus in PDB, c. Crushing of mycelial mat of the fungus, d. Drying of propagules in Ventilated cabinet dryer (Make: ITC), e. Dried propagules of the fungus, f. Grinding of the fungus, g. Addition of NaOH, h. Alkali insoluble material (AIM), i. Chitosan

Conversion of chitosan to chitosan nanoparticle

Chitosan solution (0.1%) was prepared in 1 percent acetic acid (100 ml volume). Chitosan nanoparticles were formed spontaneously upon addition of 20 ml of 1 percent tripolyphosphate (TPP) drop wise under constant stirring using magnetic stirrer (Plate 3). It was incubated under stirring condition for 30 minutes. Analysed the particle size by using DLS analyzer.

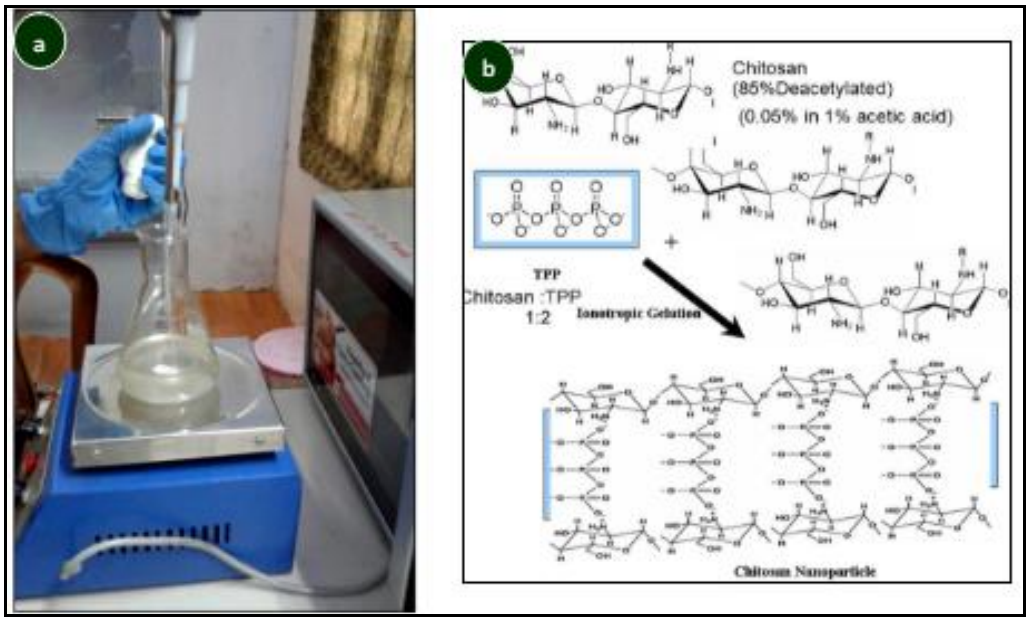

Plate 3 (a-b): Preparation of chitosan nanoparticle
a. Addition of TPP solution to chitosan solution, b. Schematic representation of preparation of chitosan nanoparticles

Characterization of chitosan nanoparticles

Characterization of chitosan nanoparticles was done by different equipment's like UV- VIS spectrophotometer, Fourier transform infrared spectrometer (FTIR), Dynamic Light Scattering (DLS), Zeta sizer and transmission electron microscopy (TEM) study).

i. UV- VIS Spectrophotometer

According to this technique many molecules absorb ultraviolet or visible light. The percentage of transmittance light radiation determines when light of certain frequency is passed through the samples. This spectrophotometer analysis records the intensity of absorption (A) or optical density (O.D.) as a function of wavelength. Absorbance is directly proportional to the path length, L, and the concentration, c, of the absorbing species. Beer's Law stated that A= € C L Where, € is a constant of proportionality, called the absorptivity coefficient. The synthesized chitosan nanoparticles were characterized by using UVV is spectrophotometer (Spectroquant pharo 300) to ascertain the formation of nanoparticle. The scanning range for the samples was 200-600 nm. Base line correction of the spectrophotometer was carried out by using a water blank reference. For the sample 2 ml was taken in a cuvette. The UV-vis absorption spectra of the sample were recorded and numerical data were plotted in the "Origin 8.5".

ii. Fourier transform infrared spectrometer (FTIR) analysis of chitosan nanoparticles

Infra-red (IR) spectra of chitosan nanoparticle recorded with a Fourier transform infrared spectrometer FTIR (Shimadzu, Japan). The spectral region between 4000 and 400 cm-1 was scanned. The spectra were the average of 50 scans recorded at a resolution of 4 cm^{-1}.

iii. Zeta potential

Zeta potential analysis is a technique for determining the surface charge of nanoparticle in solution (colloids), nanoparticle has a surface charge that attracts a thin layer of ions of opposite charge to the nanoparticle surface. This double layer of ions travels with the nanoparticle as it diffuses throughout the solution. The electric 31 potentials at the boundary of the double layer is known as the Zeta potential of the particles and has values that typically range from +100 mV to -100 mV. The magnitude of the zeta potential is predictive of the colloidal stability. Nanoparticle with zeta potential values greater than +25 mV or less than – 25 mV typically have high degrees of stability. Dispersions with a low zeta potential value will eventually aggregate due to Vander Waal inter-particle attractions. Zeta potential is an important tool for understanding the state of the nanoparticle surface and predicting the long term stability of the nanoparticle. For the analysis of Zeta potential 2 ml of the sample was taken in a cuvette and then the particle distribution in liquid was studied in a computer control charge analyser (ZETA sizer, Nano series, Malvern instrument Nano Zs, 2000).

iv. Transmission Electron Microscope (TEM)

TEM study was done at accelerating voltage of 20 kv with magnification of 20,000 X. The shape and size of the nanoparticles was elucidated with the help of TEM (JEM-2100). Aliquots of chitosan nanoparticle solution was placed on a carbon-coated copper grid and allowed to dry under ambient conditions and TEM image were recorded.

v. Dynamic Light Scattering (DLS)

DLS which is based on the laser diffraction method with multiple scattering techniques was employed to study the average particle size of chitosan nanoparticles. The prepared sample was sonicated 3-4 times by using ultrasonicator. Than the particle distribution in sample was studied in a computer controlled particle size analyser (ZETA sizer, Nano series, Malvern instrument Nano Zs, 2000). Poly dispersity Index (PDI) is generally determined by DLS and expressed in two ways, firstly the PDI calculated by Malvern software and defined as:

PDI= Standard deviation of Dz (z average)/(Dz)2

For all particle system the z- average (Dz), volume average (Dz), and the number average (Dn) diameter is measured by DLS using Zetasizer Nano ZS (Malvern Instruments) PDI is dimensionless with value between 0 and 1, which is scaled such that values with 0.10 or less are considered highly monodispersed and values close to 0.07 are rarely seen. Secondly, by DCS expressed as Dv/Dn (Dv= Dw) assuming the particles have an equal density regardless

Results and Discussion

Synthesis of chitosan nanoparticles

Synthesis of chitosan nanoparticles was done from fungal source which was selected based on the presence of higher amount of chitin in the cell wall of those fungus. Selected fungus were *Fusarium oxysporum* (Plate 1). Chitin was extracted from these fungal sources and extracted chitin was converted to chitosan by following the method of Aghdam (2010) [2]. One commercial source of chitosan (Sigma Aldrich) (Plate 1) was also used for comparison. Synthesized chitosan was converted to chitosan nanoparticle by following the method of *Calvo et al.* (1997a, b, c) [3-5].

Characterization of biosynthesized chitosan nanoparticles (NPs)

i. UV- Vis spectrophotometer analysis

UV- Vis spectroscopy of synthesized chitosan NPs was carried out at a range of wavelength of 200-600 nm and results showed maximum absorption at critical wavelengths for a particular nanoparticle. In the present study, characteristic absorption band was observed for the synthesized chitosan NPs from fungi *T. asperellum* at 342.00 nm (Fig 1) respectively. Chitosan NP synthesized from commercial product of chitosan showed absorption peak at 330.25 nm (Fig 2). This UVV is spectroscopy study confirmed the formation of nanoparticles.

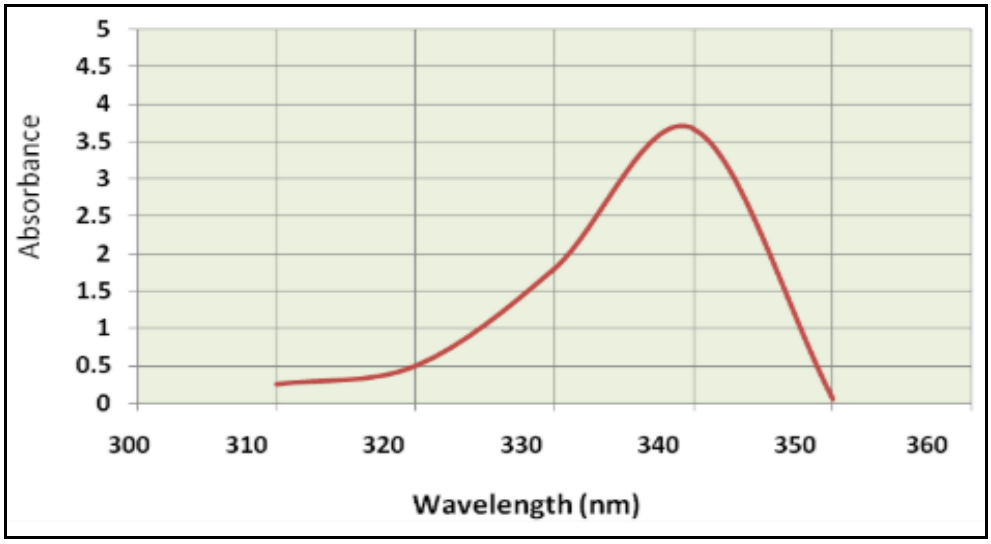

Fig 1: UV – VIS absorption spectra of synthesized chitosan nanoparticle -04 (*Trichoderma asperellum*)

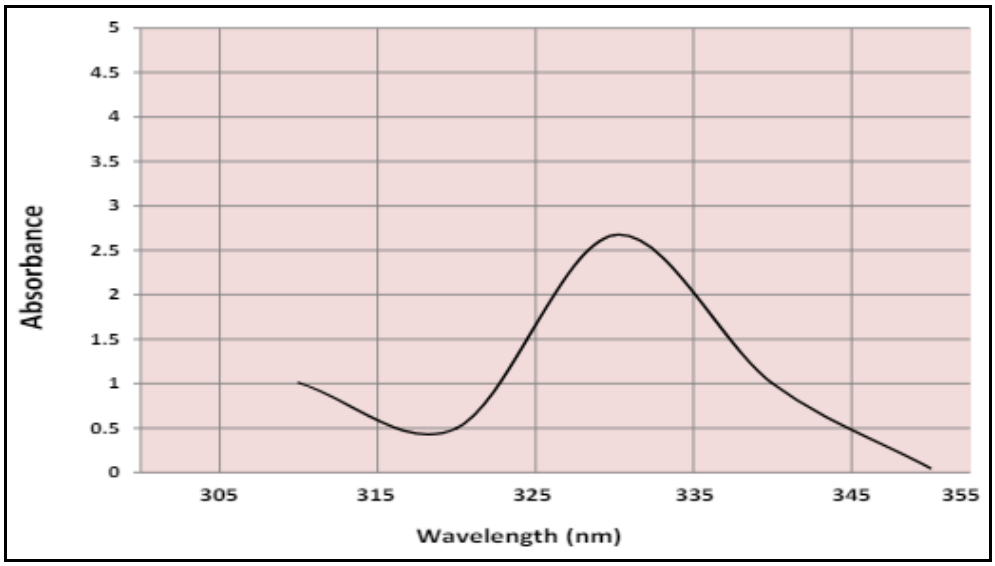

Fig 2: UV - VIS absorption spectra of synthesized chitosan nanoparticle -05 (commercial product of chitosan)

This study confirms formation of nanoparticles. Similar results were reported by AbdElhady (2012) [1] who observed absorption peak of chitosan nanoparticles at 356 nm at 40 °C of preparation temperature, and lower absorption bands of 348 and 353 nm was observed when the preparation temperature was increased to 60 °C and 80 °C respectively. But absorption peak for chitosan NP synthesized from commercial product of chitosan (Sigma Aldrich) observed at 330.25 nm.

ii. Fourier transform infrared spectrometer (FTIR) analysis

FTIR study of synthesized NPs was done to confirm the formation of chitosan NPs by identifying the required functional groups of chitosan. The main functional groups of chitosan nanoparticle are hydroxyl group (OH), amide group (NH), C-H stretching, C-N and C-O stretching and P=O stretching etc. (Plate 3b). FTIR results of synthesized NPs showed a strong and broad peak was observed at 3250 cm^{-1} (3200-3600) of the spectra, which was attributed to hydrogen bonded O-H stretching and N-H stretching vibration (Fig 3). Another peak found at 2865 cm^{-1} which was for C-H stretching (2850-3000). The peak for asymmetric stretch of C-O was found at nearer region of around 1182 cm^{-1} (1210-1320) and the peak at 1642 cm^{-1} (1550-1640) belongs to the C=O stretching (1670-1820) and N-H banding. The cross linked chitosan also showed a P=O peak at 1182 cm^{-1}. These results had been attributed to the linkage between phosphoric and ammonium ion. Presence of these required functional groups in the synthesized nanoparticle confirmed that synthesized nanoparticle was chitosan NP.

Mohammadpour *et al.* (2012) [10] reported similar results in chitosan spectra that is strong and wide peak in the 3500-3300 area, which they reported to be attributed to hydrogen-bonded O-H stretching vibration. The peaks of N-H stretching from primary amine and type II amide are in the nearer region. The peak for asymmetric stretch of C-O-C was found at around 1150 cm^{-1} and the peak at 1317 cm^{-1} belongs to the C-N stretching vibration of type I amine. Sharma *et al.* (2013) [18] reported that the peak of OH (3200- 3600) becomes wider indicating that hydrogen bonding is enhanced in chitosan nanoparticles. Similarly, Parida *et al.* (2013) [13] also observed a broad peak at 3433 cm^{-1} in case of chitosan nanoparticles. The characteristic absorption peak of chitosan was observed at 3000-3500 cm^{-1} (OH, NH2). The peak for asymmetric stretch of C-O-C was reported to found at around 1150 cm^{-1} and CH stretching at 2874 cm^{-1} (Nallamuthu, 2015) [12]. In the FTIR spectra of chitosan nanoparticles, the intensities of amide band were observed clearly. The board peak appeared at 2500 cm^{-1}, which confirmed the presence of NH3+ in the chitosan nanoparticles (Sahab *et al.*, 2015) [14].

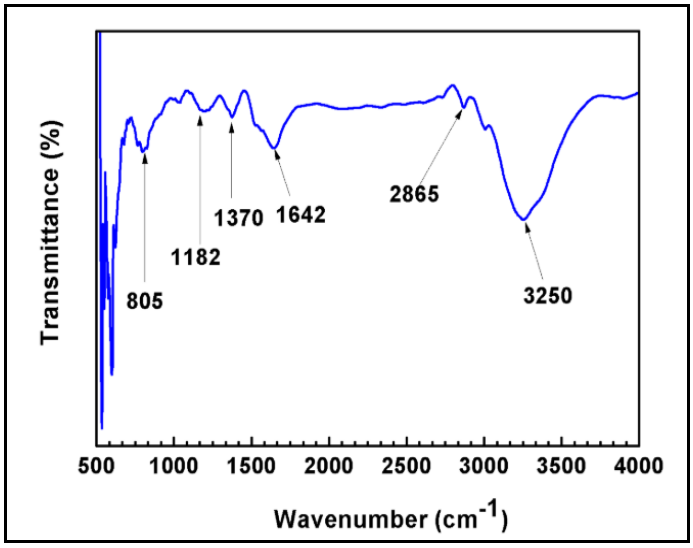

Fig 3: FTIR analysis of chitosan nanoparticle

iii. Zeta potential

As nanoparticles are small in size and are energetically very unstable in nature. Therefore, the particles undergo agglomeration or aggregation to stabilize themselves. So, there are some potential charges on the surface of nanoparticles which make them stable. To study the surface properties of chitosan NPs zeta potential values was recorded with the help of the zetasizer (ZETA sizer, Nanoseries, Malvern instrument Nano Zs, 2000). Results showed that chitosan NPs synthesized from *T. aasperellum* and commercial product of chitosan (Sigma Aldrich) were found to be positively charged with zeta potential of 9.78 (Fig 4) and 17.6 mV (Fig 5) respectively. Since the zeta potential values of synthesized NPs were recorded in the range of ± 30 mV, they are considered to be stable in nature. Besides, since chitosan NPs synthesized from and T. viride (Fig 4) were found to have zeta potential values of less than +10, they have the tendency to coagulate faster as compared to NPs synthesized from commertial product of chitosan (Fig 5).

Similarly, Nayak *et al.* (2014) [20] recorded zeta potential of +10 to +30 of chitosan NPs. The result on zetasizer analysis of the present study is in agreement with work done by Nagarajan *et al.* (2015) [11] who found that zeta potential values of chitosan NPs were positively charged and the values ranged from 11.2±1.2mV to 18.7±0.4mV. Reports says that particle charge is a stability determining factor and a zeta potential of ± 30 mV is ideal for a physical stability of any suspension (Nallamuthu, 2015) [12].

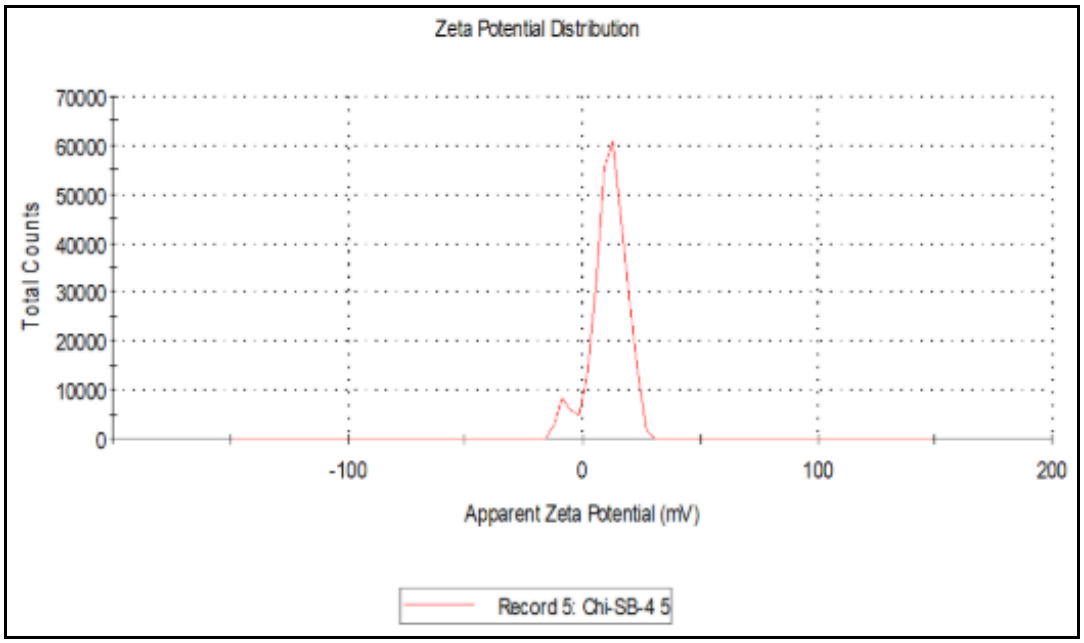

Fig 4: Zeta potential analysis of chitosan nanoparticle (*Trichoderma asperellum*)

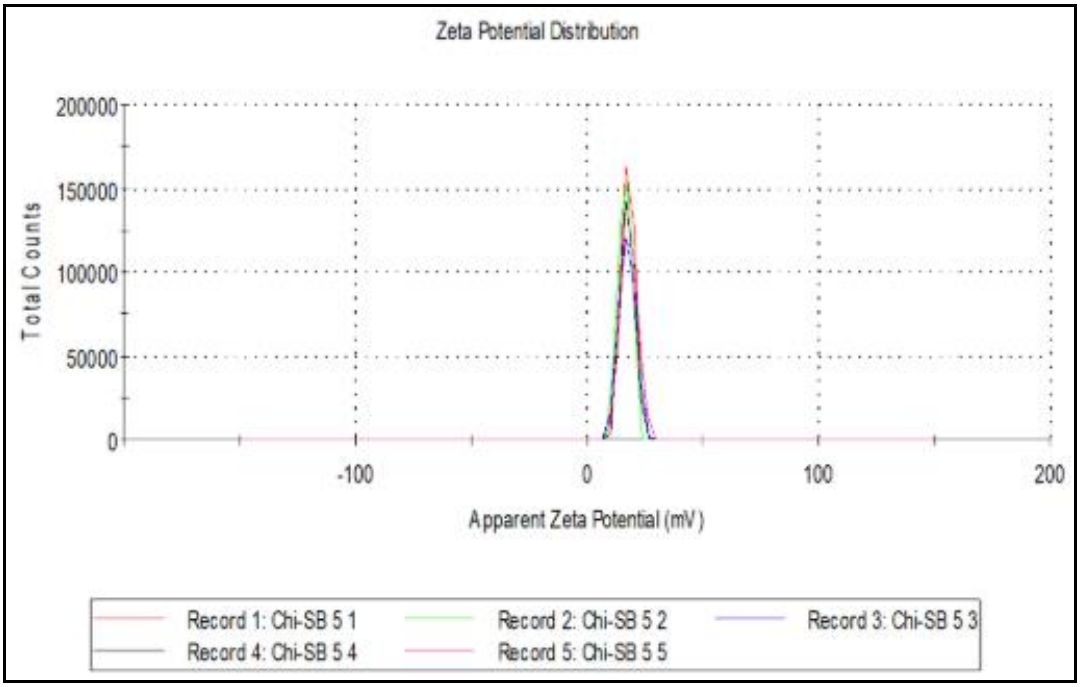

Fig 5: Zeta potential analysis of chitosan nanoparticle (commercial product of chitosan)

iv. Transmission Electron Microscopy (TEM) study

The study on shape and size of nanoparticles are two very important efficacy determining factors as because smaller size and higher surface area means higher efficacy of nanoparticles. To determine the size and shape of NPs TEM (JEM2100) was used where analysis was done at an accelerating voltage of 200 kv with 20,000 X magnification. TEM study revealed that synthesized chitosan NPs was nearly spherical in shape (Plate 5a-e). Further the electron diffraction (ED) pattern indicated that synthesized chitosan NP were amorphous in nature (Plate 5f). Similar to the present study, Feng *Qi et al.* (2005) [7] also found that chitosan NPs with dense structure and had spherical in shape. Similarly, Tao *et al.* (2011) [19] and Sharma *et al.* (2013) [18] also reported that chitosan nanoparticles were nearly spherical in shape, and the external surfaces appeared to be smooth in nature. Parida *et al.* (2013) [13] studied morphology of bare chitosan nanoparticle by SEM and reported to be of spherical to round in shape with a size of about 78 nm in diameter.

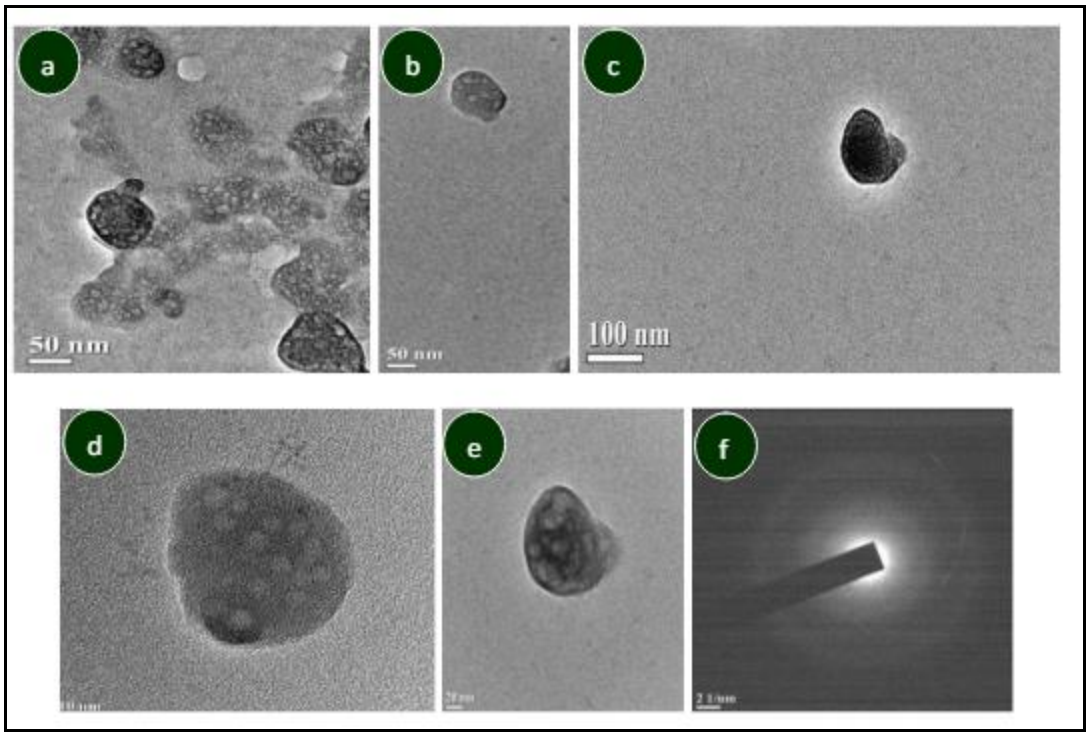

Plate 5 (a-f): TEM micrograph of chitosan NPs

a. Chitosan NP-01, b. Chitosan NP-02, c. Chitosan NP-03, d. Chitosan NP-04, e. Chitosan NP-05, f. Electron diffraction pattern of chitosan NP

v. Dynamic light scattering (DLS) analysis

Dynamic light scattering (DLS) is a technique used in material science for determination of the size distribution profile of nanoparticles in suspension or polymers in solution. This technique was used in the present study to determine the appropriate size of nanoparticles. DLS also determines polydispersity, hydrodynamic sizes and aggregation of particles in the suspension. DLS analysis done for the chitosan NPs in the present study showed that the size of chitosan NPs synthesized from *T. asperellum* and commercial product of chitosan (Sigma Aldrich) were of 89.03 (Fig 6) and 300.1 nm (Fig 7) respectively. In the present investigation, it was observed that chitosan NPs synthesized from *T. asperellum* and commercial product of chitosan were polydispersed in nature with polydispersity index of 0.308 and 0.932 respectively. Dave *et al.* (2013) [6] characterized HCl loaded chitosan nanoparticles and found the size of the nanoparticles as 210 nm. Nagarajan *et al.* (2015) [11] developed chitosan based polymeric nanoparticles with the size of 360±12nm. Similarly, the mean particles size of chitosan (CS)-g-poly (acrylic acid) (PAA) nanoparticles was found to be around 50 nm by *Sahab et al.* (2015) [15].

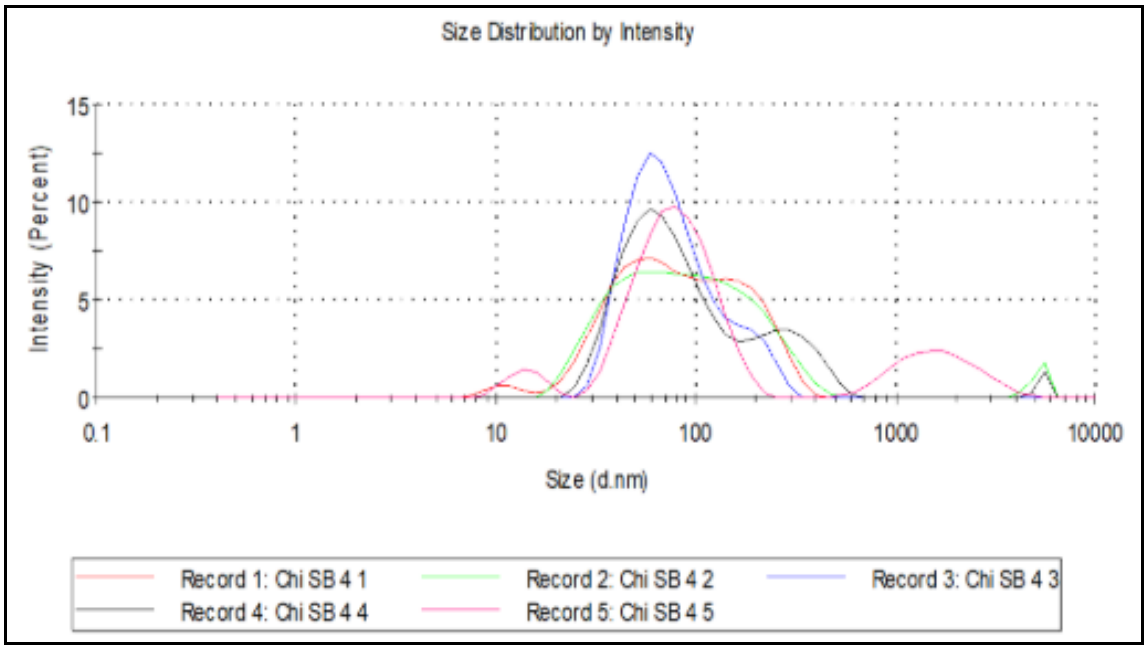

Fig 6: Dynamic light scattering (dls) analysis of chitosan nanoparticle-04 (*Trichoderma asperellum*)

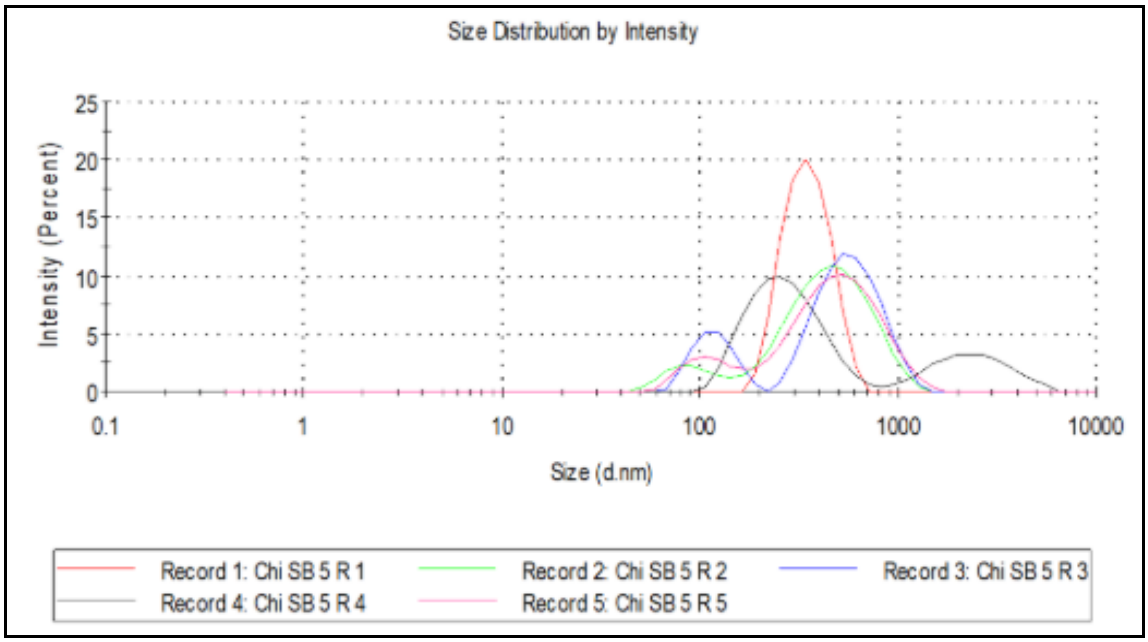

Fig 7: Dynamic light scattering (DLS) analysis of chitosan nanoparticle-05 (commercial product of chitosan)

Conclusion

This study showed encouraging result of combine use of nanoparticle and biocontrol agent for the management of soil borne plant pathogens like *Fusarium oxysporum*, *Sclerotium rolfsii* and *Rhizoctonia solani*. It opens up the possibilities of reduced use of synthetic pesticides which ultimately reduced the pesticidal pressure on the environment. Nano-bioformulation prepared by using biological sources of nanoparticles are potential organic pesticide which could be an important component of "Organic Agriculture". With suitable nano-bioformulation it is certain that it will continue to perform a major role in integrated pest management (IPM).

Future prospects

1. Future research work can be done to see the impact of chitosan nanoparticles on environmental matrix and on soil properties
2. Study need to be done on effect of nanoparticles on metabolomic activity of host plant as well as on biochemical changes in the plant system
3. Determination of cost involved in the preparation of nano-bioformulation and calculation of C: B ratio as well as shelf life study of nano-bioformulation is need to be done for commercialization of the formulated product
4. Standardization of best suitable delivery methods of nano-bioformulation

References

1. Abdelhady MM. Preparation and Characterization of Chitosan/Zinc Oxide Nanoparticles for Imparting Antimicrobial and UV Protection to Cotton Fabric. Int J Carbohydr Chem. 2012;2012:1-6.
2. Aghdam MG. Extraction of chitosan from fungal cell wall by sulfuric acid Studying the effect of deacetylation degree and temperature on recovery chitosan. Masters thesis. University of Boras. School of Engineering; c2010. p. 56.
3. Calvo P, Remunan-Lopez C, Vila-Jato JL, Alonso MJ. Chitosan and chitosan/ethylene oxide propylene oxide block copolymer nanoparticles as novel carriers for proteins and vaccines. Pharm Res. 1997a;14:1431-1436.
4. Calvo P, Remunan-Lopez C, Vila-Jato JL, Alonso MJ. Novel hydrophilic chitosan-polyethylene oxide nanoparticles as protein carrier. J Appl Poly Sci. 1997b;63:125-132.
5. Calvo P, Vila-Jato JL, Alonso MJ. Evaluation of cationic polymer coated nanocapsules as ocular drug carriers. Int J Pharm. 1997c;153:41-50.
6. Dave R, Patel R. Preparation and characterization of doxorubicin HCl loaded chitosan nanoparticles by w/o emulsion method. Int J Pharma Life Sci. 2013;4(2):2407-2413.
7. Feng Qi L, Xu Z, Li Y, Jiang X, Han X. In vitro effects of chitosan nanoparticles on proliferation of human gastric carcinoma cell line MGC803 cells. World J Gastroenterol. 2005;11(33):5136-5141.
8. Kwon JH, Park CS. Stem Rot of Tomato Caused by Sclerotium rolfsii in Korea. Mycobiol. 2002;30(4):244-246.
9. Mauch F. Diseases of fruits and vegetables- Diagnosis and management. In: Naqvi SAMH, editor. Kluwer Academic Publishers. New York, Dorddrecht. 1988;2:521-523.
10. Mohammadpour DN, Eskandari R, Avadi MR, Zolfagharian H, Mir Mohammad Sadeghi A, Rezayat M. Preparation and in vitro characterization of chitosan nanoparticles containing *Mesobuthus eupeus* scorpion venom as an antigen delivery system. J Venom Ani Toxins Inclu Tropi Dis. 2012;18(1):44-52.
11. Nagarajan E, Shanmugasundarama P, Ravichandirana V, Vijayalakshmia A, Senthilnathanb B, Masilamanib K. Development and Evaluation of Chitosan Based Polymeric Nanoparticles of an Antiulcer Drug Lansoprazole. J Appl Pharma Sci. 2015;5(4):20-25.
12. Nallamuthu I. Chlorogenic acid loaded chitosan nanoparticles with sustained release property, retained antioxidant activity and enhanced bioavailability. Asian J Pharm Sci. 2015;10(3):203-211.
13. Parida UK, Rout N, Bindhani BK. In vitro properties of chitosan nanoparticles induce apoptosis in human lymphoma SUDHL-4 cell line. Advan Biosci Biotechnol. 2013;4:1118-1127.
14. Sahab AF, Waly AI, Sabbour MM, Nawar LS. Synthesis, antifungal and insecticidal potential of Chitosan (CS)-g-poly (acrylic acid) (PAA) nanoparticles against some seed borne fungi and insects of soybean. Inter J Chem Tech Res. 2015;8(2):589-598.
15. Sahab AF, Waly AI, Sabbour MM, Nawar LS. Synthesis, antifungal and insecticidal potential of Chitosan (CS)-g-poly (acrylic acid) (PAA) nanoparticles against some seed borne fungi and insects of soybean. Inter J Chem Tech Res. 2015;8(2):589-598.
16. Saikia P. Pathogenecity of Fusarium oxysporum f. sp. Cubense and effect of a few antagonists on it. Masters thesis. Department of plant pathology, AAU, Jorhat; c2004. p. 135.
17. Saremi H, Okhovvat SM, Ashrafi SJ. Fusarium diseases as the main soil borne fungal pathogen on plants and their control management with soil solarization in Iran. African J Biotechnol. 2011;10(80):18391-18398.
18. Sharma S, Sharma U. Synthesis, characterisation and determination of encapsulation efficiency of chitosan nanoparticles for terbinafine. Indo Ameri J Pharmaceuti Res. 2013;3:1564-1567.
19. Tao Y, Zhang H, Gao B, Guo J, Hu Y, Su Z. Water-soluble chitosan nanoparticles inhibit hypercholesterolemia induced by feeding a high-fat diet in male sprague-dawley rats. J Nanomaterial. 2011;2011:1-5.
20. Nayak D, Roth TL, McGavern DB. Microglia development and function. Annual review of immunology. 2014 Mar 21;32:367-402.