

ABSTRACT

Seeds are regarded as the basic foundational element of healthy agricultural system. But these seeds, during post-harvest storage period are distressed by broad-spectrum of pathogens, generally called seed-borne pathogens. Majority of the seed contaminants are fungal pathogens that causes seed-borne diseases of economically important staple food crop like wheat. Infection caused by seed-borne fungal pathogens not only laid the seed failure for germination, but also, hinder the seed quality and its nutritional status. Thus, management of seed-borne fungal pathogens is essential for the appraisal of wheat agriculture.

Nanotechnology has offered new dimensions for the management of plant diseases through the adaptation and modification of some specified biological elements. One such specialized, versatile biological element is chitosan, the deacetylated product of fungal cell wall component – chitin. Chitosan is an established plant disease healer and growth promoter. Its alteration of structural configuration with the implementation of essential transitional metal ions, those that possess antimicrobial properties and required by plants as micronutrients, resulted in the formation of bioactive metallic nanoconjugates of chitosan. Hence, this research is based on the management of selected post-harvest seed-borne fungal pathogens of wheat through the application of metallic nanochitosan.

This research includes the synthesis of five different metallic nanochitosan, involving, Ag, Cu, Ni, Zn and Fe – nanochitosan for the management of high incidence post-harvest seed-borne pathogens of wheat. Metallic nanochitosan were synthesized by following Ionic Gelation Method and characterized on the basis of UV-vis spectrophotometry, FE-SEM, HR-TEM, DLS, zeta potential, FT-IR, etc. Initially, seed-borne fungal pathogens were isolated from five (Sonalika, PBW 343, DBW 187, HD 2967 and HI 8759) different wheat cultivars, available locally in regions of North Bengal. Ten seed-borne fungal pathogens were isolated through

blotter paper and agar plate method, of which six high incidence fungal pathogens, including, *Aspergillus flavus*, *A. niger*, *Rhizopus stolonifer*, *Fusarium solani*, *Penicillium chrysogenum* and *Curvularia pallescens* were selected for the *in vitro* antifungal application of metallic nanochitosan. *In vitro* antifungal assay resulted Ag-nanochitosan (Ag-CNPs) at 500 µg/mL as the most potential antifungal metallic nanochitosan in compromising the fungal spore germination rate and terminating the mycelial growth of high incidence seed-borne pathogens. Among all the synthesized nanoparticles, Ag-CNPs produced severe irreversible membrane damages on the spore membrane of the selected fungal pathogens resulting in the failure of spore viability. Ag-CNPs resulted in the generation of maximum oxidative stress in the fungal system and high amount of fungal lipid peroxidation by the estimation of malonaldehyde (MDA) %.

In vivo application of Ag-CNPs, Cu-CNPs and Ni-CNPs on wheat seeds through solid matrix nano-priming technique revealed that Ag-CNPs efficiently enhanced the seed germination % and vigour index followed by the enhancement of seed storage proteins (albumin, globulin, gliadin and gluten) and carbohydrate composition (starch, soluble and insoluble sugar) of the tested wheat cultivars. In addition, Ag-CNPs promoted the antioxidant enzymes, such as, CAT, POD, SOD, NOX, etc., in seed through nano-priming technique, despite of pathogenic interference. Pathogenic stress on the seeds were generated through artificial inoculation of the high incident seed-borne pathogen – *A. flavus*. Seeds were exposed to spore suspension of *A. flavus* post-priming for 6 h. Appraisal of the antioxidant enzymes in the seed elicit the disease resistance ability by lowering the disease incidence % and fungal load on the nano-primed wheat seeds. Seeds nano-primed with effective dosimetry of Ag-CNPs successfully withstand pathogenic stress as reflected in plant height and root length stress tolerance index. Furthermore, Ag-CNPs nano-priming on five wheat cultivars resulted in increased vegetative growth parameters of the seedling along with the enhanced biochemical attributes such as,

protein, phenol, chlorophyll, reducing sugar, proline, etc. Ag-CNPs nano-priming on five cultivars of wheat seed uplifted the antioxidant defensive enzymes required for resistance against pathogen.

Assessment of disease incidence on five wheat cultivars revealed Sonalika as the most resistant cultivar, DBW 187 as moderately susceptible and HI 8759 as the most susceptible cultivar against *A. flavus* seed-borne pathogen. Thus, further studies were carried out in order to uplift HI 8759 from susceptibility towards resistance. SDS-PAGE studies on total protein profiling of the nano-primed seeds revealed Ag-CNPs nano-primed seeds with more intensified bands. The elicitation of resistance in stressed seeds was further confirmed by Native-PAGE analysis of defensive enzymatic isoforms of CAT, POD, SOD, PPO, etc. Cytotoxicity analysis of all the synthesized metallic nanochitosan was studied on mammalian HEK 293 and ACHN cell line. It was revealed that the applied dosimetry of metallic nanochitosan used for seed nano-priming was totally non-toxic to mammalian cell line.

Moreover, Ag-CNPs nano-priming in the three selected cultivars: Sonalika (highly resistant), DBW 187 (moderately susceptible) and HI 8759 (highly susceptible), resulted in increased yield parameters, in comparison to non-primed seeds. Post-harvest management was carried out by nano-priming of the wheat seeds with 500 µg/mL Ag-CNPs, soon after they were harvested and stored in air-tight bags before sowing. Examination of fungal load of the nano-primed seeds revealed that Ag-CNPs primed seeds germinated successfully, while, non-primed seeds resulted in the emergence of fungal contaminants from the seeds which failed to germinate. LC-MS analysis of the germinating seedling of the susceptible cultivar HI 8759 nano-primed with Ag-CNPs and distressed with *A. flavus* at the same time revealed the presence of 55 essential metabolites that might helped the seed to overcome susceptibility and pass the seed to a successful germination regime. On the other hand, the non-primed distressed seed inoculated with *A. flavus* produced only 29 metabolites failing to seed germination and

cannot overcome susceptibility. Henceforth, this research on the management of wheat seed-borne pathogens suggests the use of Ag-nanochitosan as the most potential fungicidal agent and crop yield promoter. Ag-nanochitosan played dual function of an antifungal agent and plant growth promoter as well.