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Full Length Research Paper

Fortification of wheat grains during storage against fungal contamination of aflatoxins by coating seeds with zein-zinc coordination complex

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Cereals are important foods which provide the bulk of our dietary requirements. They are also sources of carbohydrates, which are metabolized by our body for energy generation. Cereal grains undergo huge storage loss, which is significantly due to fungal contamination; on the other hand nutrient deficiency also coexists. The spoilage mainly occurs due to moisture absorption during storage leading to fungal growth at high temperature and humidity, so, grain moisture is the key for fungal contamination to occur. In the current work, coating technique with zinc coordinated zein (Zinc@Zein) nano-layer was used as a viable strategy for long term grain storage of wheat (*Triticum aestivum*). Zein can prevent moisture flux and zinc can provide anti-microbial property. Zinc was spread on the solid grain surface as a nano-scale film. The nano-layer of Zinc@Zein on *Triticum aestivum* provided efficient protection from seed borne pathogen of *Aspergillus flavus* and *Pseudomonas Syringae* infection with a reduction of 65% and 80%, respectively, in the seed borne compared to control. Thus the zinc coordinated zein with wheat was proven to improve the efficient protection of seed-borne pathogens.

Keywords: Mycotoxins, Aflatoxin, Coating seeds, Antifungal, Wheat storage.

INTRODUCTION

Grains are the major energy source for human and they also act as the seed for propagation (Kumar and Kumar, 2017). In cereal grain, 50 to 60% is lost during storage. Mostly due to microbial contamination, which deteriorate the nutrient value and cause a quarantine issue (National Advisory Committee on Microbiological Criteria for Foods, 1999). Fumigation is the widely practiced method to avoid microbial contamination of the stored grain, which often show residual effect, hence biocompatible plant smoldering was developed recently (Tayel, 2010). Chitosan and other coatings were also employed for seed protection from microbial contamination (Reddy et al., 1999). Since, the coating solution was prepared with water, it is limited for the seed treatment just before sowing and not compatible for pre-storage processing. For a long term storage of wheat, yeast coating was reported, but this was found to change the chemical composition of wheat (Harman, 1983).

Stored grain infection is known to happen through four ways; i. natural or manmade openings in the grain; ii. Hilum; iii. Micropyle; and iv. grain accessary structure like hair. Furthermore, for transition the fungi do not need to internally infect the grain, but the surface is sufficient. The grain infections were specifically proportional to the relative humidity (RH) (Darsonval et al., 2008). Major part of the world still store the grain in ventilated open go-down (Kumar and Kumar, 2017), so the grain moisture is often fluctuated to maintain equilibrium. Agricultural pathogens, like *A. flavus and Pseudomonas syringae*, were found to be the most important grain contaminant, which can disseminate even by air (Cottyn et al., 2001). Further the wheat seed infected with

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Pseudomonas syringae were known to cause 50% infection in the germinating plants, irrespective of resistance and susceptible variety (Fryda and Otta, 1978).

Hence an edible hydrophobic moisture barrier coating at the grain level is desired. Zein with hydrophobic to hydrophilic surface area of 3.8:1 that can be dispersed in ethanol could be a perfect match (Hurley and Prud'Homme, 2016). But the challenge is to get continuous coating, since even for planar surface coating, plasticizers were used with zein. However plasticizer like the oil may increase stickiness eventually favoring infection (Soliman et al., 2009). Non-sticky plasticizer, like glycerol, compromises the water barrier property (Ying and Padua, 2004). No study has taken forward the zein coating to test food preservation. Further the reported zein films were in micron scale measuring, >120µm, 150-180µm, 79µm, 1µm, however, these were not pursued on food (Shi et al., 2011).

On the other hand, zinc deficiency is an alarming problem, and known to cause brain malfunction and compromise in immune system with physical growth impairment (Herman et al., 2002). Zinc is also an anti-microbial agent both as ions and in oxide forms (at Nano scale) that can complement grain protection (Heinlaan et al., 2010). The zein film is also proven to support the activity of the loaded active ingredients like the silver ion (Zhang et al., 2010), lysozyme (Gucbilmez et al., 2007) and antioxidant (Herald et al., 1996). This present work was designed to test the efficiency of zinc coordinated zein nano-layer on protection against fungal infection on wheat.

MATERIALS AND METHODS

Materials

Wheat, Zein, $ZnSO_4$, pepsin, bile salt, pancreatin and ethanol were purchased from TCI Chemicals. Peptone, malt extract, yeast extract, dextrose and beef extract were purchased from HIMEDIA. Sodium hydroxide, Glycerol, Tris base, SDS, Ammonium persulphate (AP), TEMED, Acrylamide, Bis-acrylamide, glycine, Bromophenol blue and Coomassie brilliant blue were purchased from SIGMA-ALDRICH. All the reagents used were of analytical or HPLC grade. The immunoaffinity columns Afla^{Test} were purchased from VICAM (USA). Other chemicals and solvents were of analytical or HPLC grade, provided via Carlo Erba (Milan, Italy). Deionized water was purified by MilliQ system (Waters, Milford, MA, USA).

Coating

Zein coating of *Triticum aestivum* grain was performed by solvent evaporation process with X% zein solution (value of "X" is mentioned in result) in 10 mL volume (9:1 ratio ethanol: water)/ 5 g of grain (for brevity this will be represented as TaS@Z). Similarly for the zinc coordinated zein composite (Zinc@Zein) coating, Zinc@Zein was prepared by incubating 1mg of ZnSO4 in 0.2% of zein in 10 mL of 9:1 ratio ethanol: water for 2 h. Any other ratio of this zinc to zein if used, is mentioned in the result. Following this, Zinc@Zein was coated on wheat grain by following the same solvent evaporation method mentioned above for zein coating. This will be represented as TaS@Z-Z.

Surface characterization

The scanning electron microscopy image of the coated and uncoated wheat grains were observed with JEOL JSM 1500 (USA). Three-D surface profile of the coated and uncoated wheat grains were measured with a non-contact optical profilometer (Model: Bruker GT-KO). Surface charge of the wheat grain before and after coating was measured with Surface Zeta Potential Cell (ZEN1020).

Fragmentation of protein

Fragmentation of protein was done by SDS-PAGE gel electrophoresis. One percent zein solution was prepared with and without $ZnSO_4$ in 80% aqueous ethanol and mixed with sample buffer in the ratio of 80:20. Sample buffer was prepared by mixing 20% glycerol, 0.5 M Tris-HCl of pH 6.8 and 0.5% bromophenol blue. Stacking gel (4% polyacrylamide, 1.5 M Tris-HCl, pH 6.8, 10% SDS, 0.1% ammonium persulphate, 0.01% TEMED) and separating gel (15% polyacrylamide, 1.5 M Tris-HCl. pH 8.8, 10% SDS, 0.1% ammonium persulphate, 0.01% TEMED) were prepared in a gel-casting plate and submersed in tank buffer (0.25 M Tris, 1.92 M glycine, 1% SDS). The gel was run at 80-120 mv and the fragmentation of protein was observed by Bio-Rad geldoc.

Circular dichorism

The secondary structure of zein protein with and without $ZnSO_4$ were analyzed by circular dichroism spectrometer (JASCO J-1500-150). Sample was prepared by dissolving zein and zinc coordinated zein in 1mL of 80% aqueous ethanol with the amount of zein been same in both the samples.

Viscosity

Viscosity of zein solution was measured (Anton paar MCR 320) at 25°C with the viscometer at spindle speed

of 100 rpm. Viscosity were recorded at different time (1 min, 7 min, 15 min, 30 min, 45 min, 60 min) after spindle rotation had been initiated. Viscosity measurement of zein and zinc coordinated zein samples were taken in 80% ethanol at the same concentrations.

Zinc estimation

The concentration of zinc was measured from the digested sample obtained from the grain flour using the inductively coupled plasma (ICP-MS).

Fourier transforms infrared spectroscopy

Fourier transform infrared (FT-IR) spectrum analysis was carried out by using the Cary 600 series FT-IR spectrophotometer (Agilent technologies). Thin peel for the grain (with and without coating) was collected, powdered and KBr pellet of the powder was prepared to collect the FTIR spectra.

Moisture dynamics

To enumerate the ability of the different coating to act as the moisture barrier, 5 g of grains were placed at 95% relative humidity at which generally the grains are prone to microbial contamination. The grains were heated at 130 °C for 1 hour and incubated in the bio incubator for different intervals up to a month with 75% relative humidity. The difference in the weight of the grain before and after incubation is calculated as percent moisture content with reference to the initial grain weight. Moisture (%) = (final weight – initial weight/ final weight) x 100.

Water resistance

To check the effect of hydrophobic zein layer in resisting water entry onto the grain, different treatment of grains were dipped in water and the water was decanted after a short period of 20 and 600 seconds incubation. Then the moisture content in the seed was estimated as above after a brief uniform air drying.

Bioavailability

The bioavailability of Zinc ions from the wheat grain, with and without coating was enumerated through the method described previously (Verran and Boyd, 2001). Briefly the powdered sample of the grain (1g) was allowed for digestion with pepsin (300mg/10 mL) adjusted to pH 2. Following the digestion an aliquot (1 mL) was taken to check the amount of NaOH required for pH makeup to pH 7.5. This titrated mole of NaOH was packed in a dialysis tube in the remaining aliquot, following this incubation for 30 minutes pancreatin–bile mixture was added for further digestion, following which the supernatant was analyzed for the zinc ions with ICP.

Antimicrobial activity

For fungal, 5 samples (50g) wheat grains had been contaminated by *A flavus* strain with initial inoculated concentration 10, 11, 9, 12 and 14 ppb each in flask. 50g wheat grain free of *A. flavus* was taken as control. Five replicates for each tested sample of wheat grain were taken; *A. flavus* strain (RCFF, ARC, Egypt). *A. flavus* were maintained on Czapek solution agar with 20% sucrose modified with 7 g per liter of Difco yeast extract. The basal medium (YES) contained 2% yeast extracts (Difco) and 20% sucrose. Demineralized water was used throughout the study. Flasks (1-liter) containing 100 ml of medium per flask were stoppered with foam plugs and autoclaved for 15 min at 20 psi-media, were inoculated with spores from 1- to 3-week-old cultures of *A. flavus* and incubated 6 to 8 days at 25 °C as stationary cultures. Following quantitatively collected and cleaned up on Afla^{Test} and results was recorded.

For bacterial, the Seed borne pathogen of wheat, *Pseudomonas syri*ngae NCIM 5102 obtained from NCIM culture collection from NCL, Pune was grown in Nutrient Broth medium at 250 RPM at 28±2 °C. The infection of the wheat seed with the bacteria was done following the standard protocol as mentioned earlier. Wheat grains (5 g) were incubated in 10 ml of bacterial culture at log phase (OD value approximately 0.6 at 600 nm), and in filtered with vaccum for 20 min and allowed for 24 hours of incubation. Following this the grain colony count was enumerated with the seed flour after serial dilution.

RESULTS AND DISCUSSION

The *Triticum aestivum* (wheat) grain was coated with the biopolymer zein protein, through solvent evaporation technique. The coating was tested with 2% zein solution in 10 mL volume (9:1 ratio ethanol: water)/ 5gm of grain. Here the solvent viz., ethanol used in the coating method is non-toxic and can simultaneously sterilize the grain. The coated samples were observed under the SEM to confirm the coating. At 2% zein concentration, the coating layer is found too thick ≥10 µm (Figure 1).

Hence, zein concentration was reduced to 0.2% and 0.02%, which resulted in merged appearances of transverse and longitudinal ridges in the control (Figure 1a and inset), confirming thinner coating (Figure 1b, 1c respectively). However, there was an incomplete surface coating in both 0.2 and 0.02% that is marked with arrows, which cause the film thickness to increase beyond 2 µm. Comparatively 0.2% coating showed

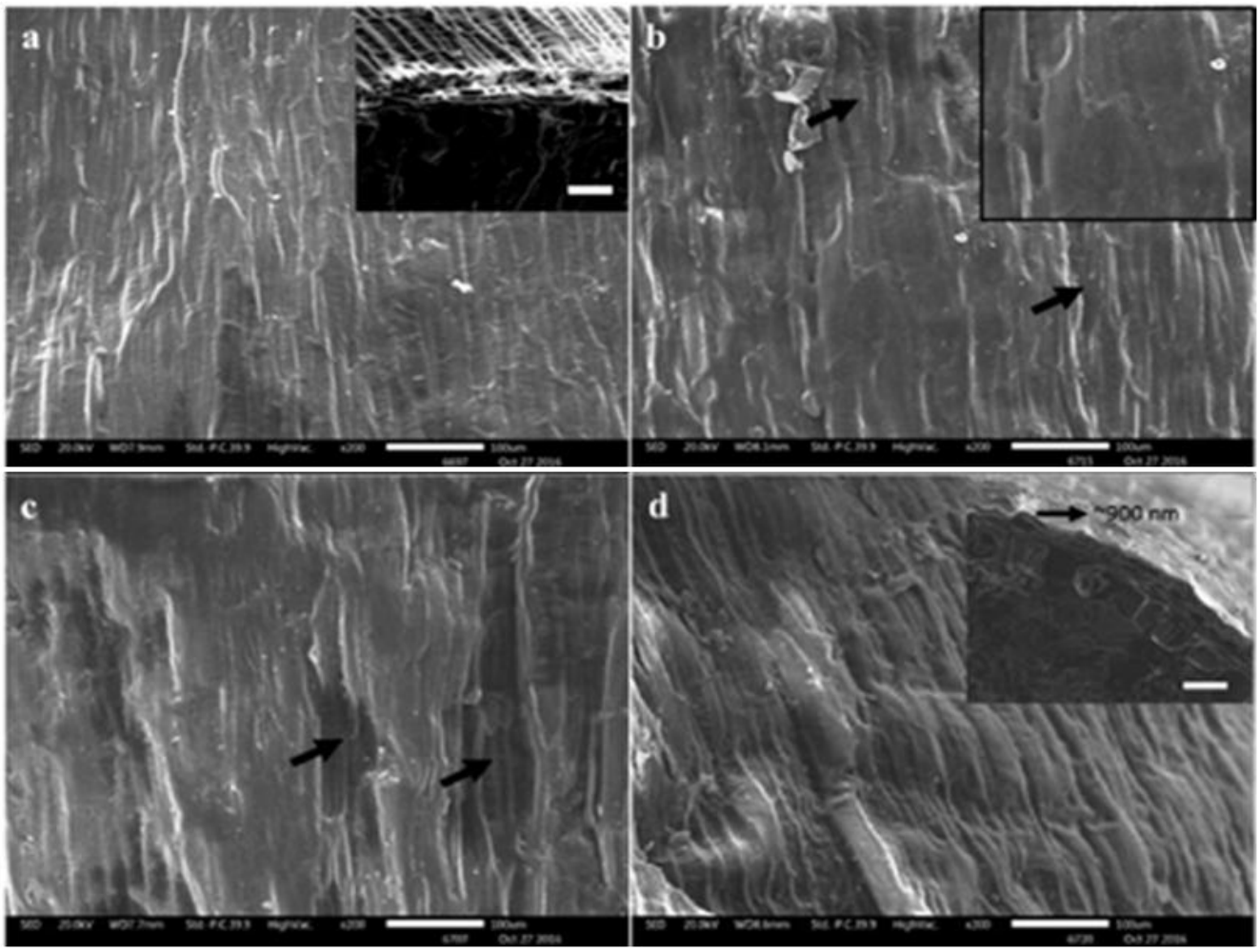

Figure 1. SEM image showing surface morphology of *Triticum aestivum* after different coating treatment (a) Control without coating (b) 0.2% zein coated (c) 0.02% zein coated (d) 0.2% zein+1mg $ZnSO_4$ coated.

maximum coverage, hence 0.2% was fixed as the optimum coating condition for further experiments. This sample was referred as TaS@Z (Zein coated Triticum aestivum) for brevity.

For the mineral (zinc) fortification in wheat grain, zinc sulphate (3mg) was added to 0.2% zein solution, followed by 1h incubation before coating. The H1NMR spectra of the zein incubated with the zinc showed peak broadening and down shift (~0.03 ppm) compared to zein in the amine peak at 8.0 ppm. The thiol peak, around 1 to 1.5 ppm, showed broadening up shifting (~0.02 ppm) after the addition of zinc. This could be due to the closer binding of zinc with the electron donors like nitrogen and thiol (Figure 2). Supporting this fact earlier reports say the metal ions bind to the protein through binding with N, O and S. This down/up shift and broadening happens due to the coordination bond formation (this composite was referred to as Zinc@Zein) (Luten et al., 1996; Miao et al., 2017). The test for zinc leaching from Zinc@Zein complex at 20 mg of zein to 1 mg of ZnSO4 ratio showed an almost negligible zinc loss; hence this ratio was followed for further studies until unless noted. Similar to zein coating, Zinc@Zein was coated on wheat by solvent evaporation, for brevity, this sample was referred as TaS@Z-Z (Zinc@Zein coated Triticum aestivum). In SEM, TaS@Z-Z coating (Figure 1d) was found to be well assembled on the grain surface compared to TaS@Z. Further the thickness of the coating was measured to be around 700 to 900 nm with the cross-section (Figure 1d inset).

Although this was not even around the grain, especially at the furrows in grain surface, the thickness was slightly more, still this is much thinner compared to the earlier zein coatings in several microns((Herald et al., 1996).. Practically, this control in the thickness gave efficient exposure to zinc ion with minimal zinc content to protect the pathogen (vide infra). The elemental zinc EDAX mapping showed TaS@Z-Z sample to have increased zinc spots compared to the control without coating (Figure 3a and b). In corroboration to the EDAX

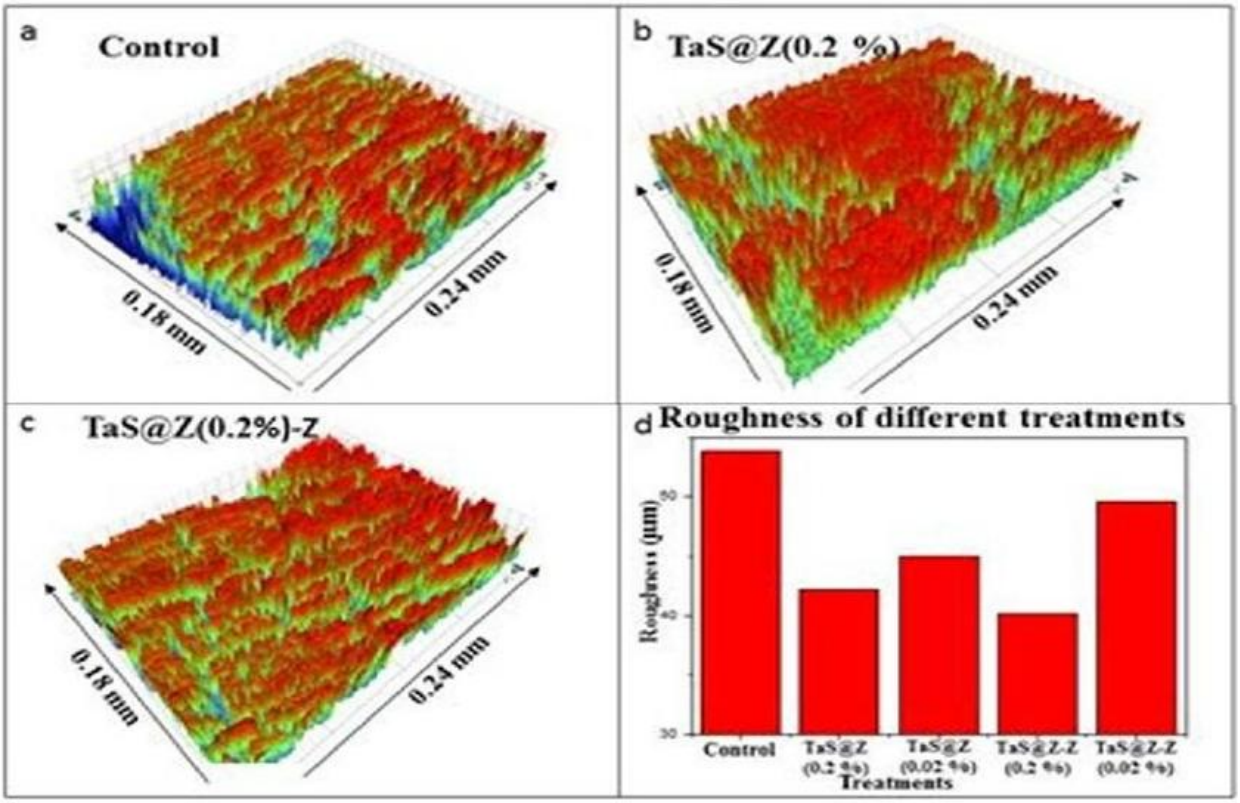

Figure 2. Surface profile of *Triticum aestivum* after different coating treatment (a) Control without coating (b) 0.2% zein coated (c) 0.2% zein+1mg ZnSO4 coated (d) Roughness plot of different coating treatments (Control: Without coating; TaS@Z(0.2%): 0. 2% zein coated; TaS@Z(0.02%): 0.02% zein coated; TaS@Z-Z(0.2%): 0.2% zein +1mg ZnSO4 coated; TaS@Z-Z(0.02%): 0.02% zein +1mg ZnSO4 coated).

mapping, the spot EDAX also showed significant increase in the zinc content in TaS@Z-Z, compared to the control (Figure 3a and b).

In addition to the coating thickness, the SEM let to note that the surface of the coated grain was smooth compared to the grain without coating. Hence, to probe this feature, the surface profile of the wheat was studied with the optical surface profiler. Profilometer can give better resolution to this kind of heterogeneous rough surface in the range of nano to micron scale. The results from the profilometer study are plotted in Figure 2. In corroboration to SEM image the profilometer shows marginal reduction in the roughness in the profile of the zein coated grain compared to the control grain. However with Zinc@Zein coating viz, TaS@Z-Z shows marginal increase in roughness to 49.5.

To understand the reason for thin coating in TaS@Z-Z, the zein was investigated for molecular weight, secondary structure, viscosity and contact angle in comparison to Zinc@Zein. The SDS-PAGE analysis confirms that the zein and Zinc@Zein both have the characteristic band at 19 and 22 kDa (Figure 3a). With a closer examination of the bands there is a slight increase in the molecular weight of the Zinc@Zein at both 19 and 22 kDa region, which may be attributed to the marginal increase in the molecular weight because of metal binding. Further absence of the band at a lower molecular weight, confirms that the interaction of zinc on zein in Zinc@Zein didn't fragment the protein. The band at higher molecular weight in zein is known to happen because of native character of zein to form dimer (Yamashita et al., 1990). The CD spectra show the typical negative maxima of α-helix structure at 207 and 222 nm (Figure 3b).

The α-helix of zein diminished with the addition of the zinc ion. This pattern may be due to the partial denaturation of zein due to the disulphide and hydrogen (amine and carboxyl group assisted) bond breakage that have participated in the coordination bond formation with zinc ion. This reduction in the α-helix may also favour a reduction in brittleness (Shi et al., 2009), hence complement the coating life. Following the CD characterisation the viscosity of zein and Zinc@Zein was compared at frequent intervals with 1 hour of incubation (Figure 3c). This incubation time is at which, most of the solvent evaporation happened in the coating experiment. As expected, in this observation the viscosity of both zein and Zinc@Zein increases with reference to time of evaporation.

By comparison among them, it was shown that there was significant control in viscosity for Zinc@Zein than zein alone. In grain coating, the samples were stirred,

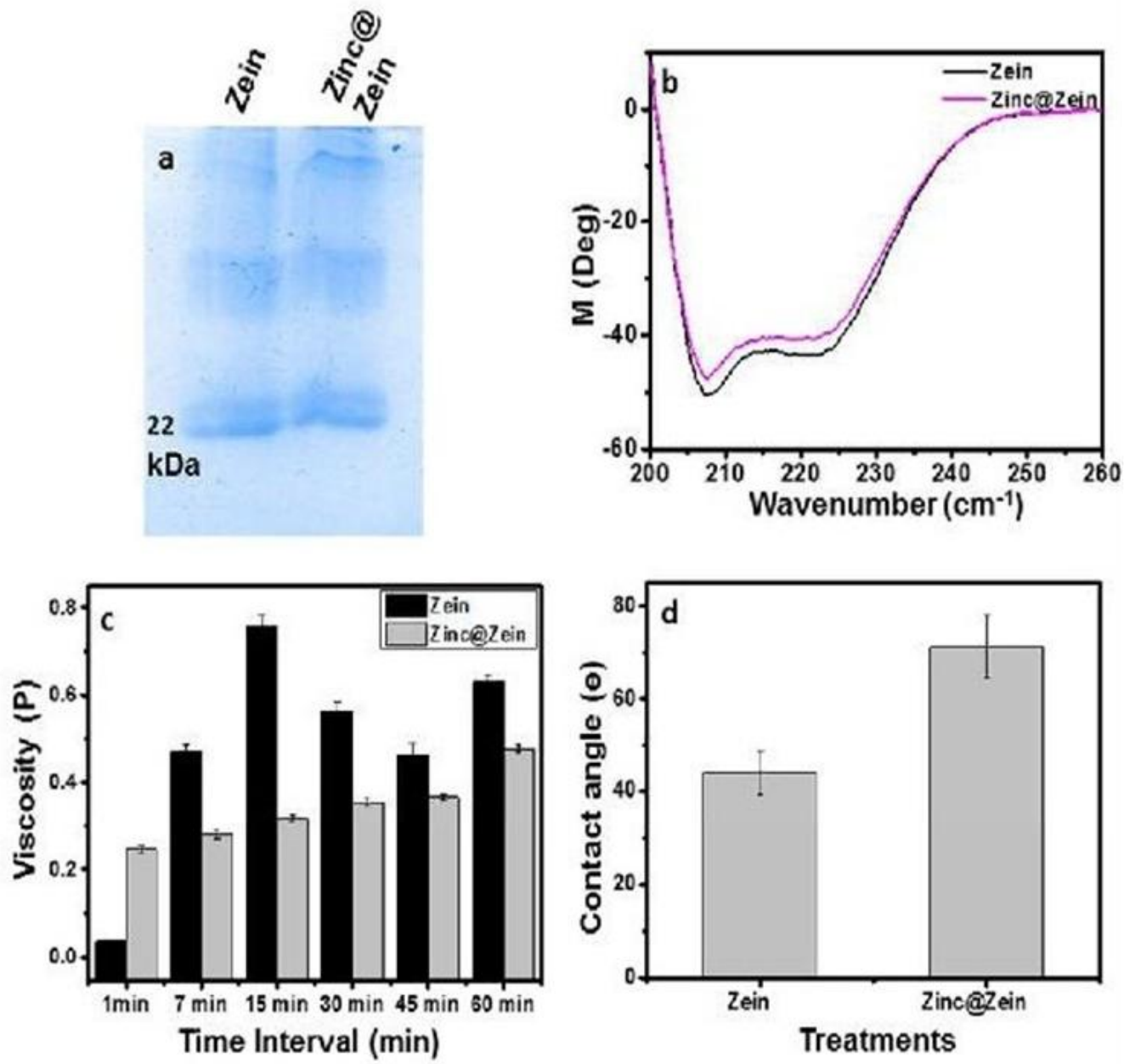

Figure 3. Characterization of Zein and Zinc@Zein coordination complex with (a) SDS-PAGE Gel documentation; (b) UV-Circular dichroism spectra ;(c) Viscosity at different time intervals of evaporation; (d) Contact angle.

hence with the increasing viscosity there was a proportional shear thickening, eventually uneven and thicker coating was evidenced in the control zein coating. Whereas, the viscosity control in Zinc@Zein may have resulted in thin and closer coating to represent the slight rough grain morphology compared to control zein coating, since such non-newtonian fluid shear thickening can be controlled by viscosity. In spite of the above modifications the hydrophobic property needs to be retained to avoid the moisture fluctuation in grain, hence the contact angle was measured (Figure 3d).

Interestingly the contact angle of the Zinc@Zein was found to be more than with zein alone, in contrast to earlier non-sticky plasticizer that reduces contact angle. This increase in the contact angle is attributed to the unwrapping of the hydrophobic domain, such exposer of hydrophobic domain with the decrease in helix was also shown earlier (Singh et al., 2009). Unlike the metal ion induced hydrophilic core and hydrophobic shell formation (Miao et al., 2017), the CD, viscosity and contact angle measurements confirms stretching of protein, which supports thinner coating.

To chemically confirm the zein coating on wheat, the FTIR spectroscopy of the ground thin sections of surface layer from the samples (control, TaS@Z and TaS@Z-Z) were measured (Figure 4a). In wheat, the outer bran

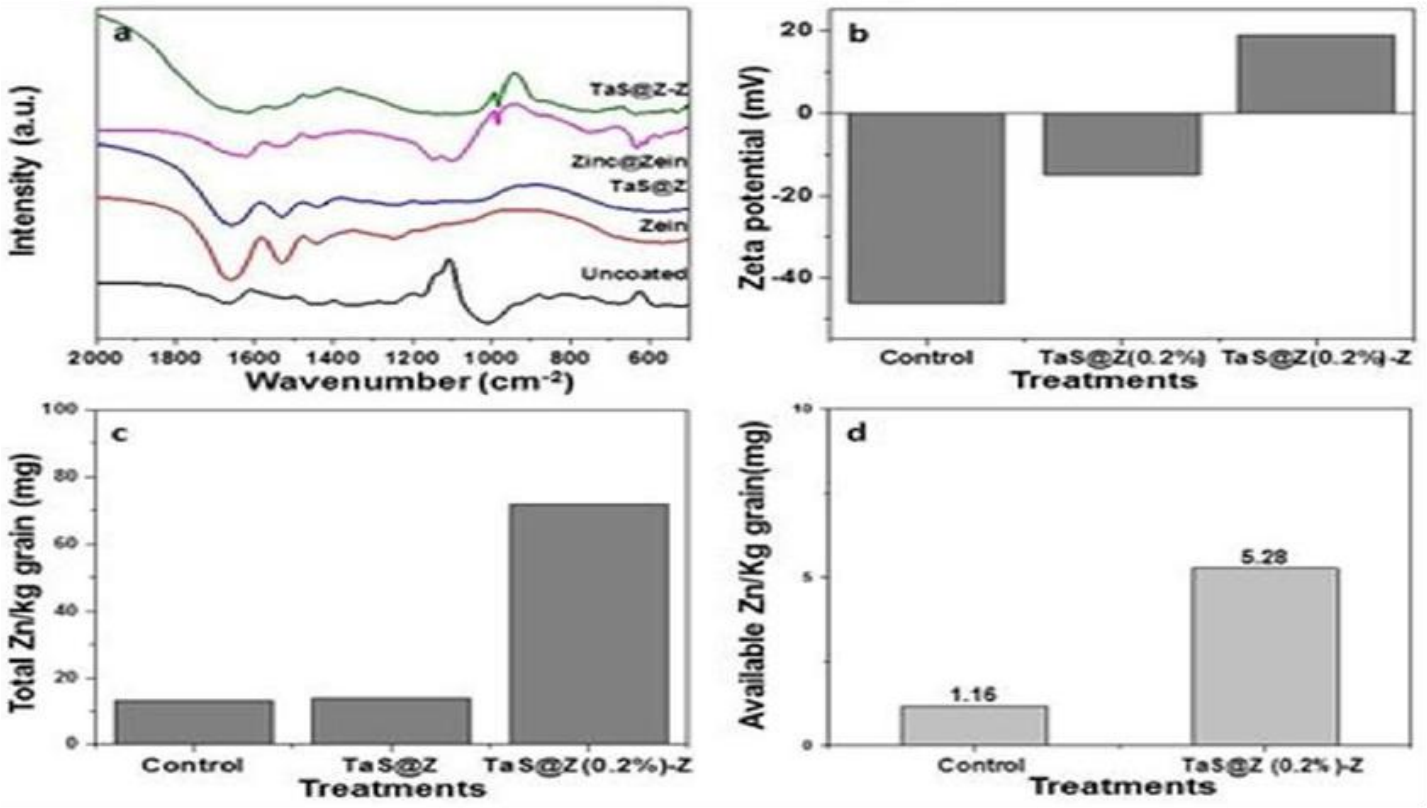

Figure 4. Characterisation of Triticum aestivum grain with different coating treatments (a) FTIR spectra of uncoated grain (surface peel), zein powder, zein coated grain (TaS@Z) (surface peel), Zinc@Zein powder, and Zinc@Zein coated grain (TaS@Z-Z)(surface peel); (b) Surface zeta potential of uncoated grain, TaS@Z and TaS@Z-Z; (c) Total zinc content in 1 kg of grain; (d) Available zinc content from 1 kg of grain.

layer extends for few micron, which is majorly contributed by the cellulose, hemicellulose and lignin (Xiong et al., 2016). The characteristic cellulose multiple peak appears for the control wheat grain outer layer at 978, 988, 1009, 1021, 1029, 1036 cm^{-1}. The wheat protein i.e., gluten is present deep inside the grain endodermis, hence protein signal at 1655 cm^{-1}, from grain surface peal is not prominent. In TaS@Z and TaS@Z-Z samples the cellulose peaks got slightly shifts to higher wavenumber, and amide 1/2 around 1655/1524 cm-1 characteristic to C=O and C-N-H are also present with slight broadening (Seyer and Gelinas, 2009; Neumann et al., 2010). Suspecting that charge could be the possible force for the wheat grain coating, the surface charge dynamics of the wheat grain before and after coating was studied. The whole wheat grain (not powdered but cut into half, the cut surface was glued in the space specified on the platform of ZEN 1020) without coating shows -46 mV. This native negative strength got reduced to -15 mV with 0.2% zein coating, which may be due to the partial neutralisation by amine group (Figure 4b). Further, when the wheat was coated with 0.2% Zinc@Zein the zeta potential raised to positive charge (+18 mV). This could be because of the binding of the zinc ion on the carboxylate group, as in glutamate of zein that causes partial negative charge in TaS@Z sample (Zhang et al., 2011). Zinc is known to form transient coordination with the protein through thiol and glutamate (Maret and Li, 2009). In support of this observation the zeta potential of the zein dispersion in 9:1 ethanol: water with pH 6.5 was measured (Figure 4). The zein shows +5 mV potential, similar weak positive charge of the zein at pH ≤10.5 was reported elsewhere (Zhang et al., 2011). This positive potential is found to be further strengthened with the zinc ion binding (~+6 mV). This proved our hypothesis that the coating is driven by charge in addition to the solvent evaporation. This degree of positive charge is known to support the horizontal arrangement of the zein rather than the vertical arrangement, which may have contributed for smoother coating here (Yamashita et al., 1990).

After the above confirmation of zein coating, the amount of zein and zinc required for coating 1 Kg of grain was estimated (Figure 4c). It was found that approximately ~2-3 g of zein was sufficient for

coating 1 kg of grain, since in 0.2% zein coating procedure standardized, almost half of the zein was found to stick to the walls of the flask. With reference to zinc fortification in 0.2% zein coating procedure, it was estimated that a maximum of ~70 mg of zinc could be fixed on the grain of 1 kg. This was ~5 times the zinc that was present in the native grain; this zinc amount fortified is within the WHO limit (Hurtado-Lopez and Murdan, 2006). Finally, the availability of the zinc from the fortified grain (TaS@Z-Z) to the control grain was compared with the simulated gut condition (Figure 4d). Here the treatment with TaS@Z 0.2% -Z showed 4.5 times increase in the availability compared to the control. The availability of zinc in TaS@Z grain is ignored as there was no change in total zinc content with reference to control. Zein was shown to have pH responsive drug release behaviour; hence it is assumed that this coating may assist in mineral release with the stimuli from the gastric enzyme (Hurtado-Lopez and Murdan, 2006).

As grain moisture is detrimental for storage, especially at 70% relative humidity microbial contamination is prone to happen (Lai and Padua, 1998), hence the samples were tested for moisture resistance. The coated samples were incubated at high RH (70% RH), and although the zein coating resisted the moisture increase it was not significant. Hence the efficiency of treatment to withstand flooding conditions was tested, by drowning the grains briefly in water. After this quick incubation time, water was drained quickly and the grain was spread on bloating paper, air dried for few minutes uniformly, before estimating the moisture content (Figure 5a). The coated samples TaS@Z and TaS@Z-Z showed less moisture compared to control, which may be due to the hydrophobic zein moisture barrier (Mudunkotuwa et al., 2012).

Anti-pathogenic effect

The Effect of TaS@Z-Z on amount of aflatoxin infection content with different culture concentration is shown in Table (1) and (Figure 6). The mean concentration values of aflatoxin in wheat seeds sample were 8, 9, 7, 10 and12 ppb for uncoated (control), 8, 9, 7, 9 and11 ppb for TaS@Z and 2, 3, 2, 4 and 6 ppb for TaS@Z-Z, respectively. The anti-microbial effect of the coating was tested with the seed borne fungi pathogen *A. flavus*, which are produce toxins (Figure 6).

The anti-microbial effect of the coating was tested with the seed borne bacterial pathogen *Pseudomonas syringae*, which are also known to produce toxins (Figure 5b). To check the efficiency of coating against *P. syringae* infection, the wheat from different treatment were allowed to incubate in the log phase bacterial broth culture. Following this the wheat samples were removed from the broth culture and the bacterial colony was enumerated. The wheat grains under TaS@Z-Z were found to show 80% reduction in the pathogen colony compared to the control and TaS@Z. But the efficiency of coating against *A. flavus* infection, the wheat grains under TaS@Z-Z were found to show proximally 65% reduction in the percentage compared to the control and TaS@Z.

Both of anti-bacterial and anti-fungal effect of the zinc oxide nanoparticles was found to be due to the release of zinc ions and this release was too less at the most favourable dissolution condition (Banerjee et al., 2011). Hence the significant reduction of infection in TaS@Z-Z may be due to the anti-pathogenic activity of the zinc, by the intracellular reactive oxygen species synthesis (Das et al., 2016), DNA damage, cationic charge and also by the imbalance in zinc homeostasis (Vicentefranqueira et al., 2015; Kara et al., 2010; Liu et al., 2014; Wu et al., 2014). In TaS@Z-Z, the zinc ions were readily available compared to the oxide nanoparticles, hence anti-bacterial and anti-fungal effect was expressed very well at least concentration. The anti-pathogenic effect could be understood better with the matching surface area of 3 nm zinc oxide nanoparticle (1.5 m^2/100 mg ZnO; ~80 mg of zinc) (Redel et al, 2011), and 1 kg wheat (1 m^2) (Marshall et al., 1984). In zein coated wheat (TaS@Z) also a slight reduction in the colony compared to the control without coating is achieved. As mentioned above, the main cause of the *A. flavus, P. syringae*, infection is hydrophilicity on the grain surface, which is overcome here by the zein coating (Tsang et al., 2007; Verran and Boyd, 2001). Next the enhancement of the smoothness with the zein treatments may have also contributed to less fungi infection, since the surface roughness aid in the microbial adhesion and growth (Redel et al, 2011). These two properties of enhanced hydrophobicity and marginal reduction in the roughness although less than the zein coating also may have complemented the anti-pathogenic effect of zinc in TaS@Z-Z.

CONCLUSION

The above coating technique; zinc coordinated zein nano-layer, can provide a viable strategy for the long term grain storage as the zein can avoid moisture flux and the zinc ions can provide anti-microbial property. Here the zinc is spread on the solid grain surface with nanoscale film, which increases the zinc surface density, hence more pathogen control at less zinc concentration. Hence this technology could help maintain phytosanitary quality in seed industry. The cost of grain preservation by this method may be nil, as there is a bonus of nutrient fortification.

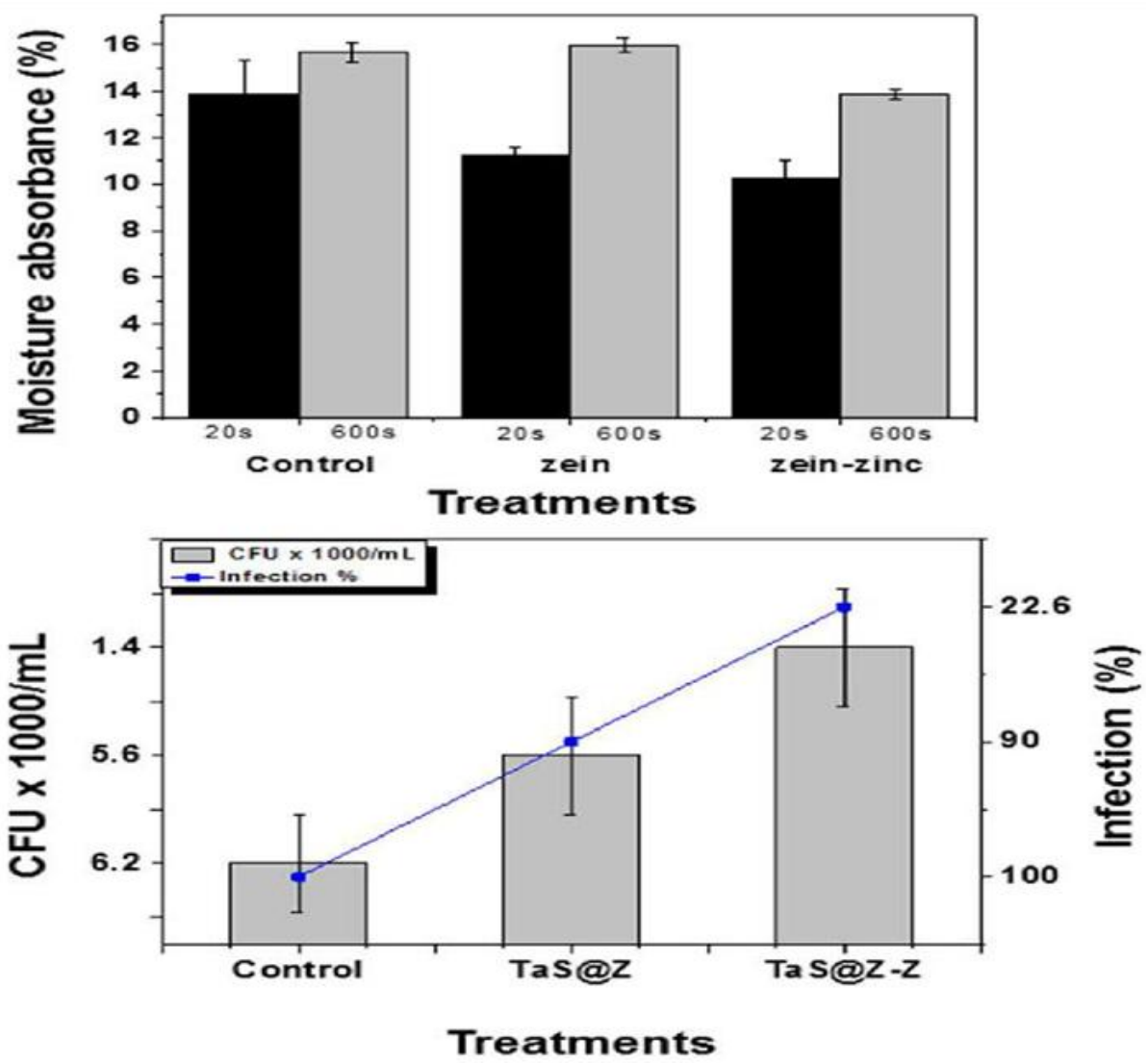

Figure 5. Efficiency of the zein and Zinc@Zein complex in moisture resistance and anti-pathogenic (a) Grain moisture content of the samples (uncoated, TaS@Z and TaS@Z-Z grain) after a brief soaking into water for 20 and 600s and followed by even air drying; (b) Anti-pathogenic effect of TaS@Z-Z in comparison to uncoated and TaS@Z by colony count method after exposing to *Pseudomonas syringae* culture at log phase for 20 min and incubation for 24 hrs.

Table 1. Contents of Aflatoxin after treatment with means were obtained using 3 replicates

Composite	Effect of coated wheat seeds with zinc coordinated zein on amount of aflatoxin infection content with different culture Conc.			
	Average conc. of culture (ppb)	Mean standard deviation	Mean conc. of culture after infection (ppb)±SEM	(%) of aflatoxin after infection
uncoated (control)	9.2	1.9235	1.84±0.8587	100
TaS@Z	8.6	1.5165	1.72±0.6770	93
TaS@Z-Z	3.4	1.6733	0.68±0.7470	36

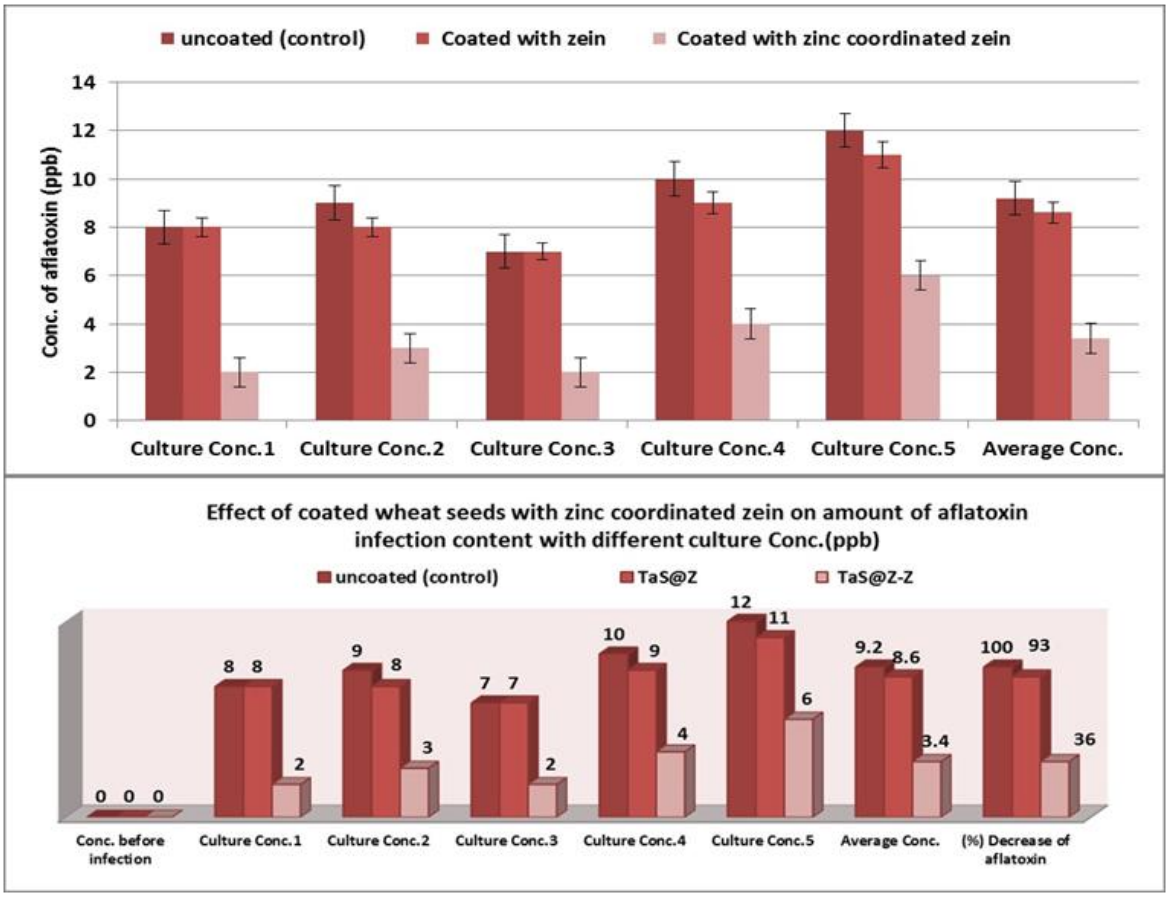

Figure 6. Anti-pathogenic effect of TaS@Z-Z in comparison to uncoated and TaS@Z with amount of aflatoxin infection content with different culture Conc (Pb).

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