Preliminary test of functionalized ZnO_2 against *Bipolaris sorokiniana* and other seed associated mycoflora for better wheat germination

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Abstract
Bipolaris sorokiniana that causes foliar blight of wheat is one of the most serious worries for growers in warmer and humid areas around the world. Use of ecologically sound molecules for management of plant diseases is considered as one of the best options to achieve sustainability of ecology, agriculture and human health. Attempts were made to synthesize PVP functionalized ZnO_2 nanomaterials to explore antifungal ability and impact on plant growth. The growth and spore germination of B. sorokiniana was significantly reduced.

The seed germination was improved with good development of plumule and radicles. Moreover, the proliferation of wheat seed associated pathogenic and saprophytic mycoflora was completely inhibited. These new informations suggest use of PVP functionalized ZnO_2 nanoparticles as an alternative to harmful fungicides for seed treatment and lower agricultural cost and environmental risk.

Keywords: *Bipolaris sorokiniana*, PVP functionalized ZnO_2 nanomaterial, mycoflora, seed germination, spore germination, *Triticum aestivum* L.

Introduction

Since wheat (*Triticum aestivum* L.) is a global staple food consumed in various forms across cultures and countries. Its sustainable production and productivity is inevitable to be maintained. Wheat can be infected with as many as 200 different diseases worldwide. Of these diseases, 42 are seed-borne and 35 are caused by fungi[1,2]. *Bipolaris sorokiniana* causes different types of symptoms viz. spot blotch, head blight, seedling blight, common root/foot rot and black point disease of wheat. However, foliar blight/spot blotch is believed to be a key disease mainly in the warmer parts of the world. Globally, around 25 million hectares of wheat have been reported to be affected by *B. sorokiniana* plant disease[3]. Indian subcontinent has 10 million hectares of affected land, out of which India alone has 9 million hectares, most of which is in the rice-wheat cropping system[4].

Foliar blight is known to reduce grain yield by 10-43% in the South Asia, especially in the Eastern Gangetic Plains of India[5-8]. It is a seed borne disease causing seed rot and reducing seedling emergence and ultimately the yield[9]. But, it can also survive in active phase on rice stubbles, where rice-wheat cropping system is common especially in South Asia[10]. However, *Hordeum vulgare*, *Avena sativa*, *Brassica campestris*, *Glycine max*, *Lens culinaris*, *Sesamum indicum*, *Vigna mungo*, *Sorghum bicolor*, *Zea mays* and *Pennisetum americanum* grown in different agro-ecological zones of wheat cultivation can serve as host of *B. sorokiniana*[11]. Like *B. sorokiniana*, the other seed borne fungi *Fusarium graminearum* and *Aspergillus flavus* have also been reported to cause germination failure[12,13].

Recently, several workers have attempted to observe the antifungal activity of several nanoparticles viz. Ag, Cu, ZnO, MgO and FeO against various fungal plant pathogens such as *Alternaria alternata*, *Botrytis cinerea*, *Colletotrichum spp.*, *Fusarium oxysporum, Rhizopus stolonifer, Mucor plumbeus, Bipolaris sorokiniana* and *Magnaporthe grisea*1[4-19]. In the present studies, functionalized ZnO_2 nanoparticles of varying sizes were synthesized in the CSIR-National Physical Laboratory, New Delhi. The details of the synthesis process have been given elsewhere[20]. The properties of nanoparticles are governed by quantum mechanics, not by other physical laws applicable to larger particles. The PVP is a biocompatible material and does not have any side effects on beings. At the same time the physical and chemical properties, biocompatibility and efficiency of functionalized material may be different from intrinsic bulk material.

There are growing concerns about the impact of nanoparticles on the environment (human, animals, plants and microbes). Hence, we carried out antifungal activity and phytotoxicity work to know impact of functionalized ZnO_2 nanoparticles. Seed germination is a rapid and widely used acute phytotoxicity test with several advantages viz. sensitivity, simplicity, low cost and stability for unstable chemicals. Therefore, to understand antifungal activity as well as phytotoxicity facts, the PVP functionalized ZnO_2 nanoparticles were tested to see its impact on spore germination, growth and spore production of *B. sorokiniana* and seed germination.

Material and Methods

Synthesis and characterization of PVP functionalized ZnO_2 nanomaterial: In the present studies, PVP functionalized ZnO_2 nanomaterials of varying size were synthesized by the reaction of zinc acetate di-hydrate with

hydrogen peroxide in ammonical water medium at 50 ± 5°C by varying concentrations of capping agents. The synthesized materials were tested for antifungal activity against *Bipolaris sorokiniana* causing foliar blight of wheat and seed associated pathogenic and saprophytic mycoflora. For the synthesis of varying sizes of ZnO_2, 10 g of zinc acetate di-hydrate was dissolved in 50% ammonia solution and further diluted to 200 ml by methanol. To this solution, 1-400% (w/w % of zinc acetate di-hydrate) of PVP was added to get desired particle size of ZnO_2. Further, equimolar quantity of hydrogen peroxide solution was added in above solution at 10±1 pH with constant stirring. The solution was vigorously stirred on magnetic stirrer for one hour after adding hydrogen peroxide solution.

The precipitates formed were centrifuged at 9000-11000 round $minute^{-1}$ speed as per size of the materials. The precipitates formed were washed several times with de-ionized water and finally 2-3 times by methanol. Thereafter, it was dried at 105 ºC in a hot air oven up to complete dryness. The synthesis of ZnO_2 materials of varying sizes was carried in de-ionized water of 18.2 MΩ-cm resistivity water prepared by USA make, Millipore milli-Q element system whereas the chemicals used for the synthesis of varying sizes ZnO_2 like zinc acetate dehydrate, ammonium hydroxide, hydrogen peroxide, PVP, TEA, PVA, MPA, methanol etc. were of analytical grade and procured from E. Merck India. The glasswares used for synthesis of ZnO_2 were procured from Borosil India Limited. The synthesis work was carried in a fume hood equipped with exhaust system.

The lab experiments were carried out using PVP functionalized ZnO_2 nanomaterials of 10-15 nm diameters. The nanoparticles of 10-15 nm settled down in water after 15-20 minutes and cannot be uniformly sprayed on the crops in the field. To take care of this problem the concentration of capping agent was increased up to ten times with respect to Zinc acetate dihydrate. Interestingly due to heavy coating of PVP over ZnO_2 surface, particles became very light and freely float in the water for more than 60 minutes. These particles were used for uniform spray over crops to find out impact on plant growth. The characterization of functionalized ZnO_2 materials of its phases in 2θ ranged from 20° to 80° and crystallite sizes were carried out using Bruker make X-ray diffraction (XRD) system, model AXS D8 Advance Diffractometer using $Diffrac^{plus}$ software. The recorded diffractions peaks corresponded to ZnO_2 (PDF # 13-0311) confirming formation of a single-phase material in each case.

The crystallite sizes of the synthesize materials were estimated by using Scherrer's equation. A representative XRD of ZnO_2 of crystallite size 10 ± 2 nm has been given in fig.1 (a). The shape and size of synthesized ZnO_2 materials were characterized using FEI, Netherland make Transmission Electron Microscopy; model F-30 G2 STWIN and LEO 440, scanning electron microscope. The TEM micrograph of ZnO_2 material of 11±3 nm diameter has been given in fig. 1(b) where as fig. 1(c) represented the SEM micrographs of another ZnO_2 material of 300 ± 5 nm diameter. Figures 1(b) and 1(c) clearly show spherical nature of ZnO_2 particles. The particles are very well dispersed in water medium and boundary of individual particles can be seen.

Isolation, purification, identification and characterization of *B. sorokiniana*: Wheat leaves infected with fungus showing typical oval to rectangular spots surrounded by yellow halo or a dark center blotch symptoms (Fig.2) were collected from Agriculture Research Farm, Banaras Hindu University, Varanasi, India. These leaves were repeatedly washed with sterilized water, dried with sterilized paper towel and finally sterilized with 1% sodium hypochlorite solution and 95% ethyl alcohol and cut into small pieces (1-2cm) by sterilized scissors. The cut leaf bits (usually four) were transferred into each of several sterilized moist chambers and incubated at 25 ± 2 ^{o}C. The spore formation took place on third day of incubation. Purification of the fungus was done by transferring a single conidium using a fine sterilized needle into each of several Petri-dishes containing Potato Dextrose Agar medium (Peeled potato: 200g, Dextrose: 20g, Agar-Agar: 20g, Distilled water: 1000 mL) acidified with 4-5 drops of 25% lactic acid.

After the formation of fungal colonies, the cultures were maintained following transfer into culture tubes containing slant of same medium in a refrigerator for further use. The fungus was characterized based on the colony characters and taxonomic features of the fungus. The colony color produced by the fungus varied from light whitish to light gray and some of them converted to black to olivacious black in color and showed presence of knotting of the mycelium. The conidia showed bipolar germination and the germ tube was in semi axial position. The number of septa varied from 3-4, but usually 3 of a 7 days old young culture while its number varied from 7-9 in older conidia.

The conidiophores were un-branched, septate, light brown to dark brown in color and erect with knee like joints. The length and width were measured for 50 spores. Their mean and standard deviation were calculated. The young conidia measured 22.6 ± 1.5 × 9.2 ± 2.8µm whereas the old conidia were 75 ± 30.5 × 18 ± 6.4 µm. These morphological characters were further confirmed with literature[21-22] and the fungus was identified (Fig. 2) as *Bipolaris sorokiniana* (Sacc.) Shoemaker [teleomorph, *Cochliobolus sativus* (S. Ito and Kurib) Drechsler ex Dastur].

Antifungal effect of ZnO_2 nanoparticles on spore germination of *B. sorokiniana*: The proposed PVP functionalized ZnO_2 nanoparticles have not been tested previously against any plant pathogen; attempts were made to test it against *B. sorokiniana* at lower concentration varying from 1 mgL^{-1} to 500 mgL^{-1}. None of these

concentrations exhibited inhibitory effect on spore germination of the fungus. Thereafter, the experiments were conducted at the higher concentrations of the ZnO_2 viz. 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000 and 10000 mg L^{-1} to see its spore inhibition efficacy. Five microliter (µl) of spore suspension was taken onto cavity slides already containing 65 µl of the different test concentrations (0.1% to 1%) of ZnO_2 nanoparticles. All the experiments were carried out in triplicates.

The cavity slides were placed in moist chambers and incubated at 25 ± 2 °C for 24 h. The sterile distilled water without ZnO_2 served as control. After 24 h, the germinated spores were seen under a compound research microscope with photographic attachment (make-Nikon UFX-II No. 230701, made in Japan) and per cent spore germination was recorded using formula as:

$$\% \text{ spores germination} = \frac{\text{No. of spores germinated}}{\text{Total no of spores examined}} \times 100$$

The pooled data were used for the statistical analysis. The collected data were subjected to the arcsine transformation prior to statistical analysis by the complete randomized design using SAS software (Version 9.2).

Effect of ZnO_2 nanoparticles on growth and spore production of *B. sorokiniana*: Under this experiment, PVP functionalized ZnO_2 nanoparticles were tested at the three different concentrations viz. 0.5, 1.0, 1.5 per cent to know impact on growth and spore production of *B. sorokiniana*. The test concentrations were added to the cool molten sterilized potato dextrose agar medium prior to its pouring into several sterilized 90 mm Petri-dishes. The composition and preparation of medium is described earlier. For each test concentration, five Petri-dishes were used as replicates. These Petri-dishes were inoculated with 5mm disc of four days young culture of *B. sorokiniana* growing in Petri-dishes. These discs were cut with the help of 5 mm cark borer from the periphery of the colony. The inoculations were done in the center of the poured Petri-dishes under aseptic conditions using laminar air flow.

The Petri-dishes without PVP functionalized ZnO_2 nanoparticles served as control. The radial growth of the fungus was measured up to six days at two days intervals. This experiment was repeated three times and the pooled data were used for the statistical analysis by the factorial complete randomized design (Factorial CRD) using SAS software (Version 9.2). The efforts were also made for microscopic observations on nature of fungal growth at periphery of the fungal colonies. The number of spores was also enumerated after making slide of the fungus growing at the various test concentrations. The slides were prepared in lacto phenol and photographs were taken using a compound research microscope with photographic attachment (make-Nikon UFX-II No. 230701, made in Japan). The measurement of 50 spores was also done and the standard deviation was computed. The data on spore production were analyzed by the Factorial CRD using SAS software (Version 9.2).

Effect of ZnO_2 nanoparticles on seed germination and plant growth: The consequences of the PVP functionalized ZnO_2 nanoparticles on growth of wheat plants were assessed following spraying of its 1.0% concentration on 21 days old plants of Agra Local variety of wheat growing in earthen pots (12 inch diameter, containing 3 kg of garden soil). Plants without spraying of test chemical served as control. The observations were taken to notice effect of ZnO_2 nanoparticles on plant growth. The effect of the functionalized ZnO_2 nanoparticles on seed germination of wheat was seen at the different concentrations viz. 0.5, 1.0 and 1.5 per cent. To accomplish this study, seeds of a susceptible wheat variety HUW-510 were used. Ten seeds were transferred into each of several Petri-Dishes containing water agar medium having 1.2% agar agar. This medium was prepared and sterilized at 15 lbs pressure for 15-20 min. The different concentrations were added during their preparation. The water agar medium without test chemical served as control.

For each concentration, five Petri-dishes were kept as replicates. These Petri-dishes with seeds were incubated at 20 °C temperature in B.O.D. incubator set for 12h light and darkness. The observations were recorded on per cent seed germination. Seed germination status was monitored six and ten days after their incubation. These seeds were also monitored and compared for the growth of the associated pathogenic and saprophytic mycoflora of wheat seeds. The associated fungi were identified following making slides and taking observation under a compound research microscope mentioned earlier. The data on per cent seed germination were subjected to the arcsine transformation prior to statistical analysis by the complete randomized design (Factorial CRD) using SAS software (Version 9.2).

Results and Discussion

A perusal of data on the effect of the various concentrations of the PVP functionalized ZnO_2 nanoparticles viz. 1000, 2000, 3000,4000, 5000, 6000, 7000,8000, 9000 and 10,000 mgL^{-1} showed varying effect of these concentrations on spore germination of *B. sorokiniana* (Table 1, Fig. 3). In the previous experiments, the test nanoparticles at their lower concentrations (1-500 mgL^{-1}) did not show any effect on spore germination of the fungus. However, in the present experiment, the spore germination was noticed to decrease with increase in the concentration over control.

The test nanoparticles showed initiation of its fungistatic activity at 1000 ppm (0.1%) but significant inhibition of spore germination was encountered at 4000 mg $^{L-1}$ (0.4%) and concentration higher than 0.4%. At this concentration only five percent spores were able to germinate in presence of the test chemical. Out of the ten concentrations tested,

complete inhibition of spore germination (P=0.05) was seen at 10000 mgL^{-1}.

The effect of the PVP functionalized ZnO_2 nanoparticles supplemented in potato dextrose agar medium at the different concentrations viz. 0.0, 0.5, 1.0 and 1.5 per cent on growth of *B. sorokiniana*, spore production and spore size varied with increase in concentrations up to six days (Table 2 and 3; Fig. 4). The radial growth of the fungus was significantly reduced with increase in concentration of ZnO_2. The growth of the fungus was nearly completely inhibited at 1.5%. At this concentration, the fungus failed to produce its spores.

The microscopic observation revealed that the functionalized ZnO_2 caused suppression of growth of the fungus at periphery of its colony due widening and coiling of hyphae. The number of spores of the fungus was also significantly reduced with increase in concentration of ZnO_2. The development of spore in term of their size was also affected. The size of the spores after six days was reduced to 17.8 ± 2.3×6.1 ± 1.0 and 17.1± 2.4 × 6.1 ± 1.2µm at the 0.5 and 1.0 percent concentrations respectively whereas it was 22.6 ± 1.5 × 9.2 ± 2.8 µm on potato dextrose agar medium without ZnO_2 nanoparticles (Fig. 4).

The data on seed germination and status of pathogenic and saprophytic mycoflora/fungal flora associated with wheat seeds during the course of seed germination in presence of the different concentrations of the PVP functionalized ZnO_2 nanoparticles viz. 0.0, 0.5, 1.0 and 1.5 percent exhibited varying effect of the test chemical on seed germination (Table 3, Fig. 5). The seed germination percentage was not significantly varied at 0.5% and 1.0% percent concentrations of ZnO_2 nanoparticles without presence of seed associated mycoflora over control. However, seed germination was better with good development of plumule and radicles at 0.5% concentration with complete inhibition of associated seed mycoflora up to six days without any adverse effect on shoot and root development over control. Conversely, seed germination was delayed with less development of plumule and radicles (roots) at 1.0 and 1.5 percent. At 1.5 percent seed germination percentage and the development of roots was also adversely affected.

The Petri-plates had no ZnO_2 showing 52% germination of wheat seeds. These seeds exhibited presence of associated pathogenic as well as saprophytic mycoflora such as *B. sorokiniana*, *Curvularia lunata*, *Alternaria tenuis*, *A. alternata*, *Aspergillus flavus, Fusarium graminearum, Cladosporium spp.* and *Penicillium spp.* However, *B. sorokiniana*, *C. lunata*, *A. tenuis*, *A. flavus, F. graminearum and Penicillium spp* were predominant. The development of roots was affected in presence of the aforesaid fungal species and led to death of radicles as well as at the base of young shoots. The absence of seed mycoflora on seed treated with the PVP functionalized ZnO_2 nanoparticles clearly unveiled the broad antifungal activity of the test nanoparticles. Conversely, the test nanoparticles were also found suitable for healthy wheat germination.

It is important to manage fungal diseases economically in sustainable manner for better human life and environment. In this connection, more efforts have been made to find out new innovative safe methods which are friendly to ecological balance and human kind and animals. This investigation on effect PVP functionalized ZnO_2 nanoparticles on *Bipolaris sorokiniana* and seed germination of wheat clearly revealed that it has antifungal activity against *B. sorokiniana* and seed associated pathogenic and saprophytic mycoflora with good impact on seed germination at 0.5%. Further, a test concentration of 4000 ZnO_2 mgL^{-1} and higher were found inhibitory to germination of the spores of *B. sorokiniana* causing foliar blight of wheat. However, the complete inhibition of spore germination was encountered at the 10000 mgL^{-1}. Similarly, the growth of the fungus was also significantly affected and nearly completely checked at 1.5% concentration of ZnO_2 nanoparticles.

The inhibition of spore germination and growth of *B. sorokiniana* may be attributed to morphological, structural and physiological changes including suppression of enzymes required for normal growth of the fungus. Similarly, there are several reports on antifungal activity of various nanoparticles. Various forms of silver ions and nanoparticles were tested by Kim et al[23] to examine the antifungal activity on two plant-pathogenic fungi *B. sorokiniana* and *Magnaporthe grisea* indicated that silver ions and nanoparticles had a significant effect on the colony formation of these two pathogens. Effective concentrations of silver compounds inhibiting colony formation by 50% (EC50) were higher for *B. sorokiniana* than for *M. grisea*. The inhibitory effect on colony formation significantly diminished after silver cations were neutralized with chloride ions.

Ouda[24] reported inhibitory effect of silver, copper and mixture of them against *Alternaria alternata* and *Botrytis cinerea*. He stated reduction in total protein, N-acetyl glucosamine (NAG) of cell wall, lipids of culture filtrate and cell wall components. His results agreed with a previous study observing that nanometer sized silver possess different properties which might come from morphological, structural and physiological changes[25]. The antifungal effect of silver nanoparticles on some pathogenic fungi has also been observed by several workers[23, 26]. It is also observed that antifungal activity of silver nanoparticles may be due to suppression of enzymes and toxins used by the fungal pathogens for pathogenesis[27,28].

Seed germination is a rapid and widely used acute phytotoxicity test with several advantages viz. sensitivity,

simplicity, low cost and suitability for unstable chemicals[29]. However, mechanism of nanotoxicity remains unknown and it would depend on the chemical composition, chemical structure, particle size and surface area of the nanoparticles. Germanton of wheat seeds up to six days at 0.5% concentration of ZnO_2 nanoparticles was good with proper development of plumule (shoot) and radicles (roots) that may be attributed to good effect on the cell division, early seedling growth and necessary metabolic pathways. These findings corroborate with Jyothsna and Pathipati[30] who reported that silver NPs even at highest dose (0.4%) did not disturb the germination of seedlings or the growth of *Ricinuscommunis*. Similarly, the lower concentrations of ZnO nanoparticles were not harmful to the cell division and early seedling growth in onion[31].

Karimi et al[32] recorded better seed germination of silver nanosolution coated seed over fungicide treated seeds and concluded to use silver nano coating instead of using fungicides. Nano titanium oxide (TiO_2) promoted photosynthesis and nitrogen metabolism and improved growth of spinach[33].TiO_2 nanoparticles promoted the germination of lettuce (*Lactuca sativa*) seeds and wheat following its electrosprying.[34,35] The multiwall carbon nanotubes penetrated tomato seeds and increased the germination rate up to 90% over control that had 71% germination by increasing water uptake and plant biomass[36]. Similarly, Siddiqui and Al-Whaibi[37] noticed that nano SiO_2 significantly enhanced the seed germination in tomato.

At higher concentrations of 1.0 and 1.5 percent, reduced development of plumule and radicles (roots) may be due to its banding with different cytoplasmic organelles and interfere with metabolic process at that site and may exert physical or chemical toxicity for germinating seeds and might result in stress or stimuli caused by the surface, size and shape of particles[38]. Lee et al[39] found copper nanoparticles against mung bean (*Phaseolus radiates*) and wheat (*Triticum aestivum*) to be toxic and resulted in reduced seedling growth rate. Similarly, silver nanoparticles disrupted cell division process causing chromatin breakage, stickiness, cell disintegration and toxical impacts in *Allium cepa* root tip cellb[40]. In a study on effect of ZnO nanoparticles on root cells of *Allium cepa*, Kumari et al[41] reported that it puts forth cytotoxic and genotoxic effect including lipid peroxidation, decreasing of the mitotic index and increasing of the micronuclei and chromosomal aberration indexes.

Poor germination and growth of shoot and roots of untreated seed showed presence of several seed associated pathogenic and saprophytic mycoflora that may be owing to toxicity of microbial metabolites of the associated mycoflora. Similarly, Dal Bello et al[42] revealed that *F. graminearum* was associated with the seedling blight of wheat complex which reduces germination, seedling stand and yield. Bhat and Fazal[43] reported that the higher concentration of culture filtrate obtained from *A. flavus* reduced the seed germination, root and shoot lengths of wheat. *A. flavus* is also a dominant storage fungus in peanuts and causes seed rots, molding of seeds, pre- and post- emergence damping off and reduction of seed viability and seedling growth.[44,45]

Proper seed germination of wheat without growth of the associated pathogenic and saprophytic mycoflora viz. *Bipolaris sorokiniana*, *Curvularia lunata*, *Alternaria tenuis*, *A. alternata*, *Aspergillus flavus, Fusarium graminearum, Cladosporium spp.* and *Penicllium spp* clearly indicates the antifungal activity of the PVP functionalized ZnO_2 nanoparticles. Although the mechanism of antimicrobial effect of the functionalized ZnO_2 particles has not been clearly understood, but there are reports in literature that antifungal activity is principally influenced by the reactive oxygen species (ROS), hydrogen peroxide (H_2O_2), superoxide anion (O_2-), hydroxyl radicals (OH_3) and organic hydro peroxides (OHPs) generated on the surface of the material causing proton leakage, resulting instability in the radical-generating and radical-scavenging systems. It stimulates the oxidative stress condition and modification in the phospholipid of the fungal cells that leads bilayer expression of stress responsive gene as well as distortion of cell membrane.[46,47]

The cell distortion may also be caused by attachment of ZnO_2 particles with the existing -SH groups in the biomolecules to inactivate the bacteria by blockage of nucleic acid synthesis[48]. Further, microbes consist of different types of cells that are composed of many different types of molecules which consist of one or more atoms joined with one or more elements by chemical bonds. When the weak bonds break, free radicals are formed that are very unstable and react quickly with other compounds. The free radicals initially attack the nearest stable molecule and the "attacked" molecule loses its electron, becomes a free radical itself. Once ROS is generated, uncontrolled attack of the membrane lipids takes place that leads to breakdown of membrane function[49].

The proposed functionalized ZnO_2 also has several free oxygen vacancies as observed from electron spin resonance (ESR) measurement. The ZnO_2 released oxygen when mixed in water and tried to get stabilized, the free radicals further attack microbe's lipid membrane. So by increasing concentration of functionalized ZnO_2 nanomaterial, the number of free radicals increases and subsequently attacks cell membrane. The inhibitory effect of functionalized ZnO_2 nanoparticles might be attributed to attack on broad range of biological processes including alteration of cell membrane structure and functions, detrimental role on total protein, lipids and N-acetyl glucosamine (NAG) and other such components of cell wall.

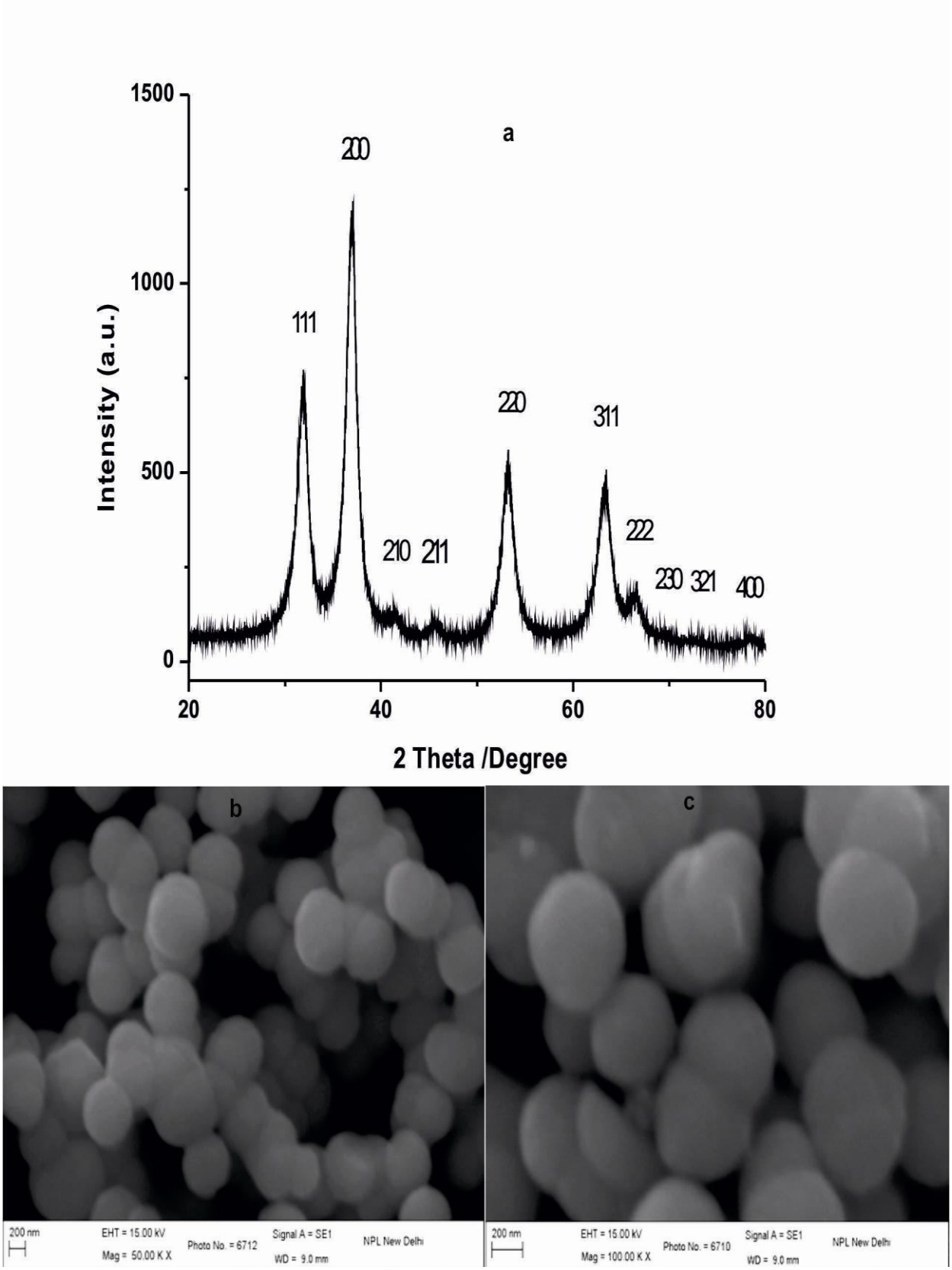

Fig.1: Illustrating characteristics features of Zinc peroxide (ZnO_2). a) XRD pattern of ZnO_2; b) SEM Micrograph of ZnO_2 at 50 KX magnification and c) SEM Micrograph at 100 KX magnification

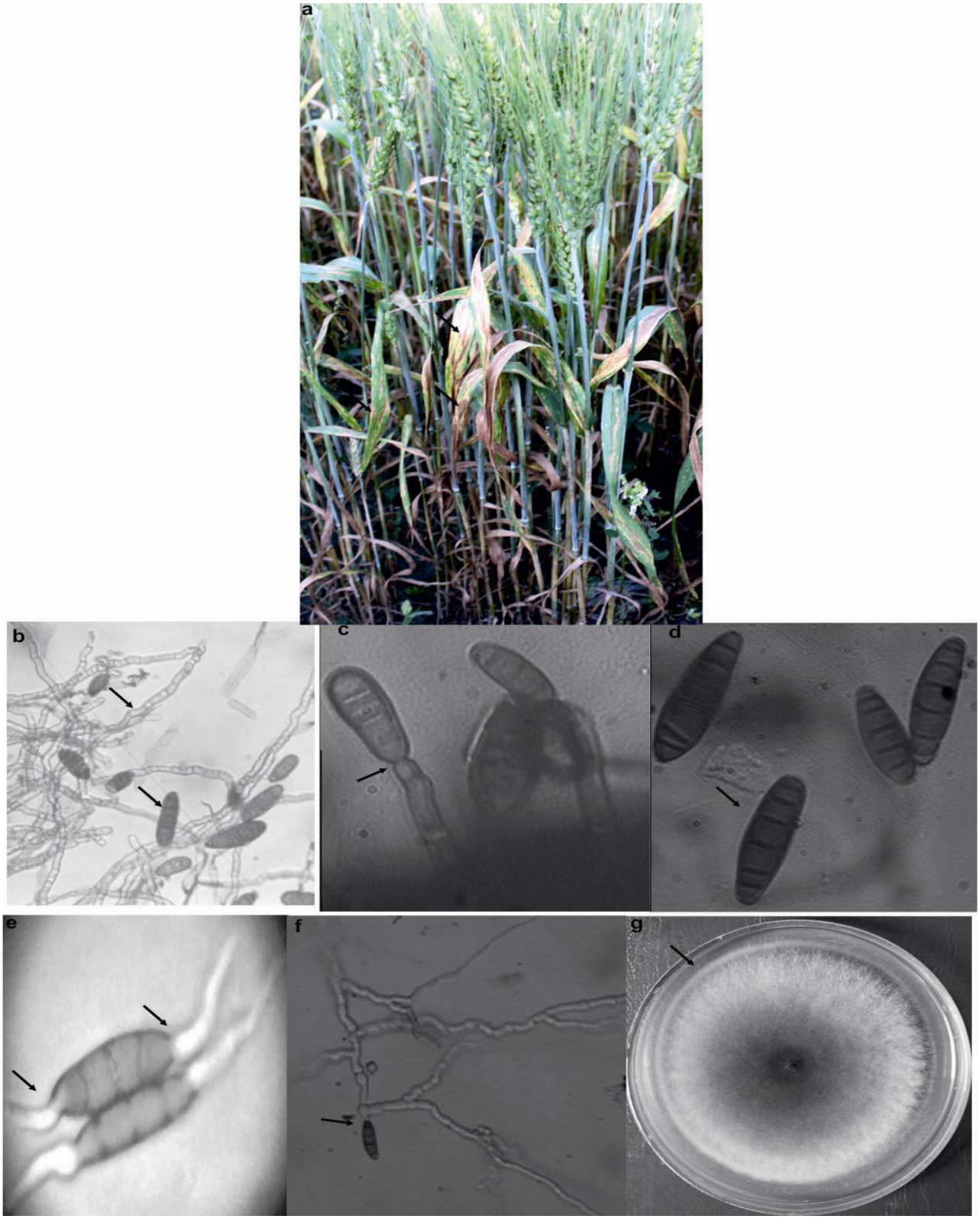

Fig.2: Illustration depicting the characteristic symptoms of foliar blight disease and its pathogen: *Bipolaris sorokiniana*. a) typical oval to rectangular spots surrounded by yellow halo or a dark canter blotch symptoms b) mycelia and conidia c) formation of new conidia on conidiophore at knee joints d) close-up view of old conidia with more than three septa e) young conidia showing semi-axial bipolar germination f) colony initiation from a single spore and g) developed colony from a single spore.

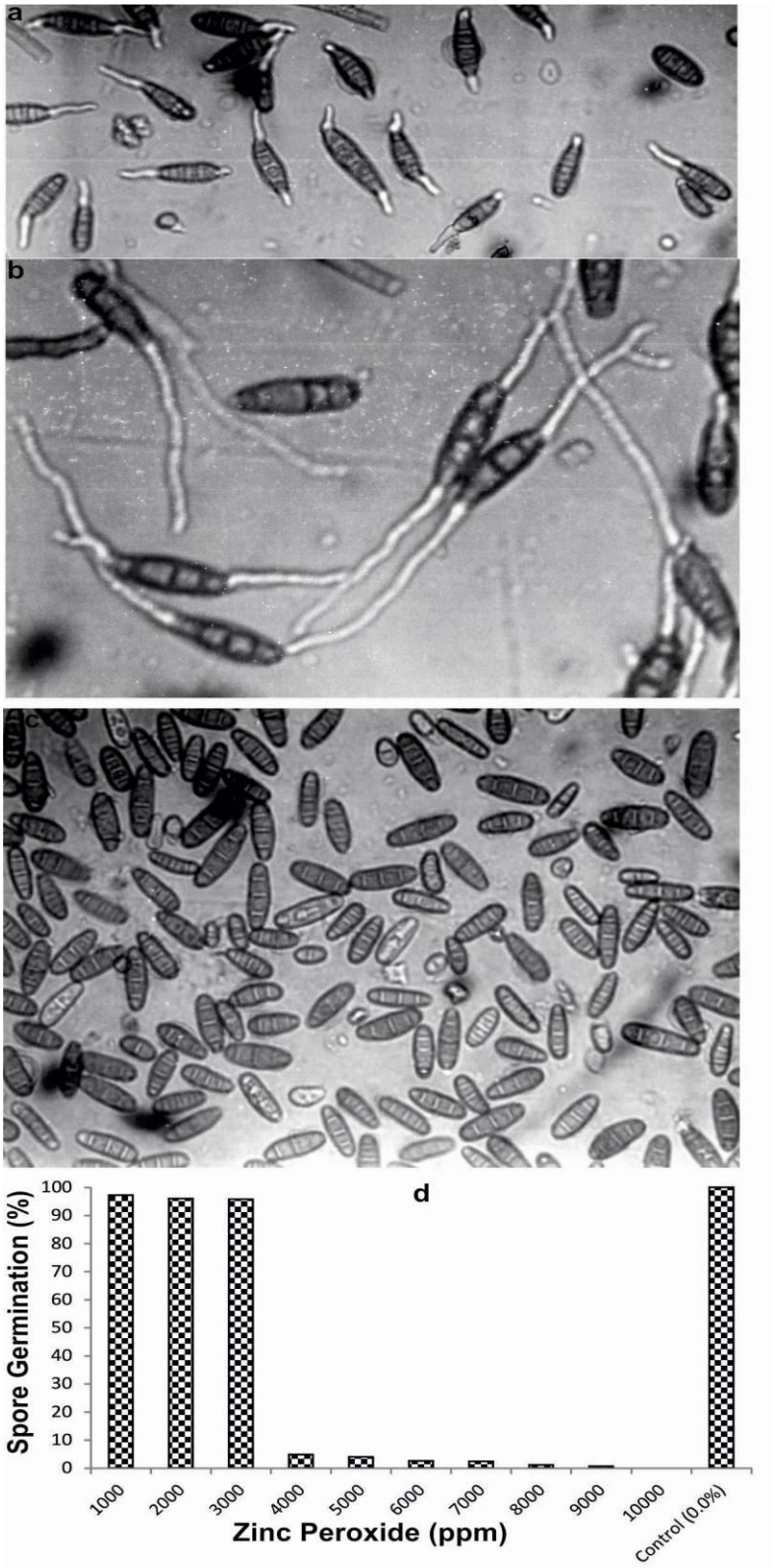

Fig.3: Viewing effect of functionalized ZnO_2 on spore germination of *B. sorokiniana a)* 100% germination in untreated control without ZnO_2 after 24h b) 100% germination in untreated control without ZnO_2 after 48h c) complete spore germination inhibition at 10,000 ppm solution of ZnO_2 and d) bar diagram representing effect of the different concentrations of ZnO_2 on spore germination

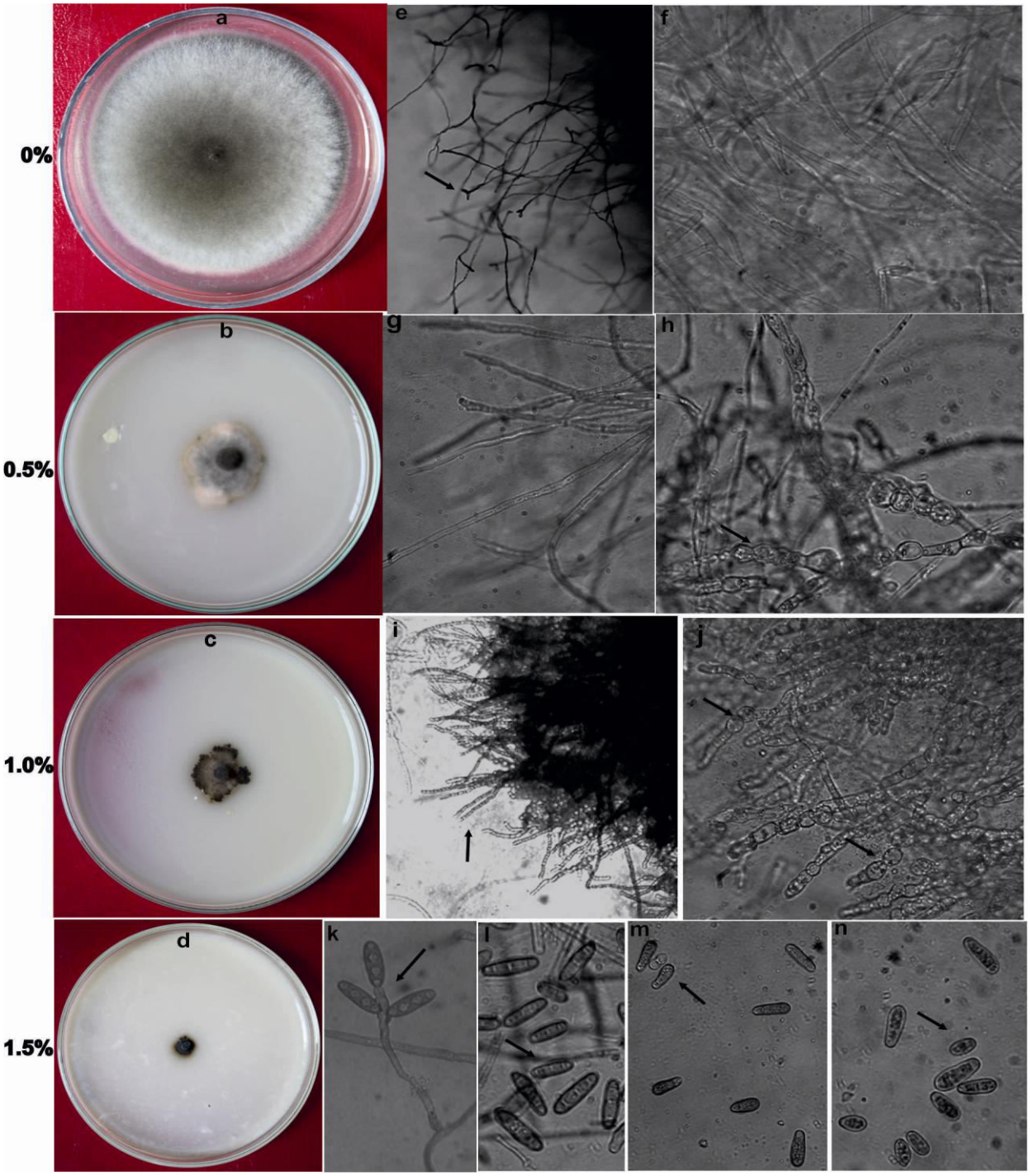

Fig.4: Presenting effect of the different concentrations (0.0, 0.5, 1.0 & 1.5%) of Polyvinylpyrrolidone functionalized zinc peroxide (ZnO_2) nanoparticles supplemented in potato dextrose agar medium six days after inoculation on growth of *Bipolaris sorokiniana* causing foliar blight of wheat and its spore size; a-d depict significant reduction in growth of the fungus with increase in concentration varying from 0.0-1.5%; e-f depict free normal growth of the fungus at the periphery of fungal colony showing attachment of spores; g-h indicate suppressed fugal growth due to widening and coiling of hyphae with few spores; i-j show excessive beading and coiling of hyphae at 1.0% concentration; k-l exhibit normal size abundant six days young spore with three septa at 0.0% of zinc peroxide and m-n show reduction in spore size at 0.5 and 1.0% of zinc peroxide.

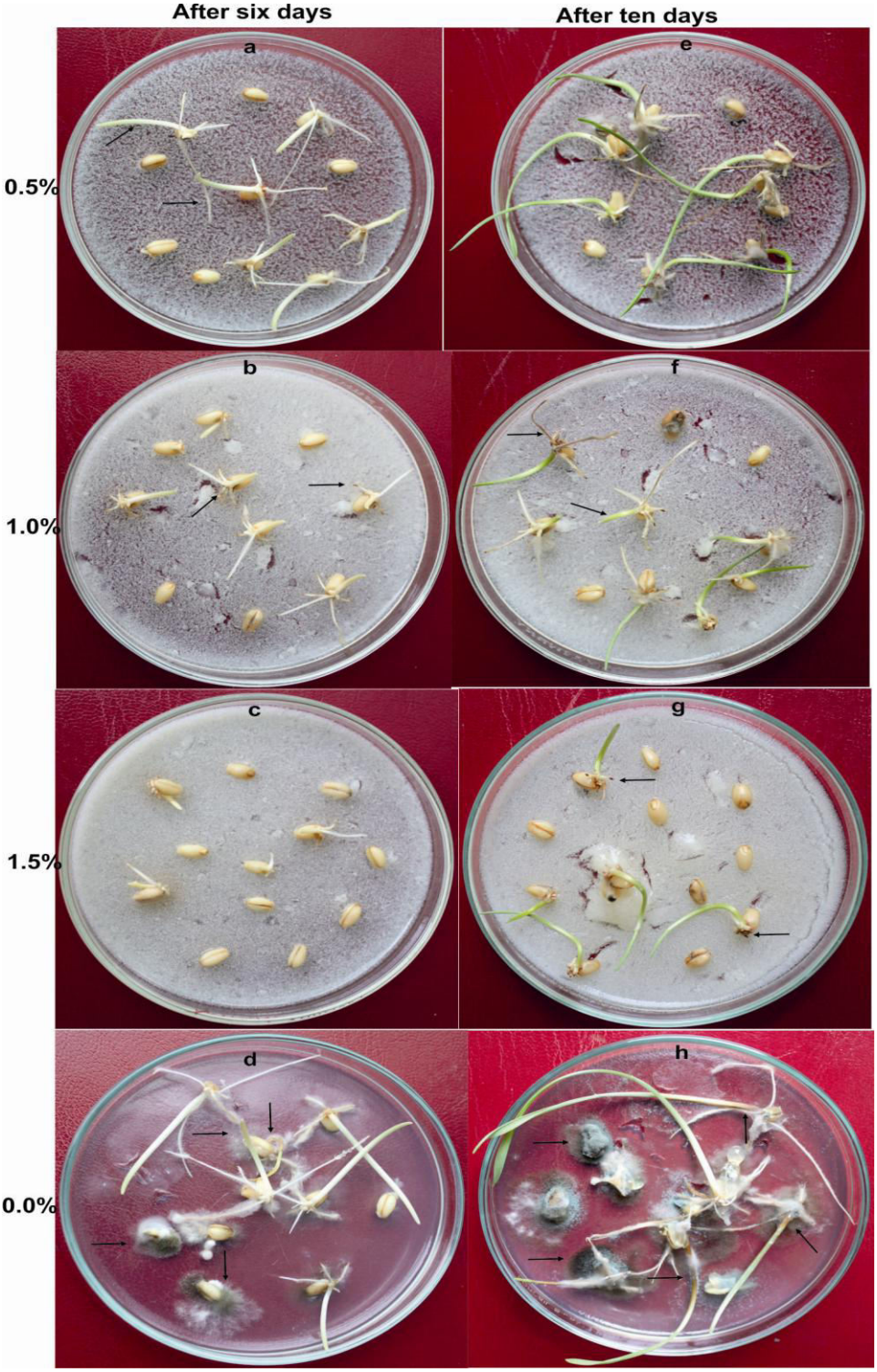

Fig. 5: Showing effect of the different concentration of Polyvinylpyrrolidone functionalized zinc peroxide (ZnO_2) nanoparticles supplemented in water agar medium on wheat germination, its quality and mycoflora associated with wheat seeds. a-d show germination of the seeds after six days of incubation at 0.5, 1.0, 1.5 and 0.0% concentrations of zinc peroxide; e-h indicate germination of seed after 10 days at 0.5, 1.0, 1.5 and 0.0% concentration; a) good germination at 0.5% concentration without growth of associated fungi; b-c and f-g indicate delayed germination without fungal growth and poor development of roots; d and h indicate poor germination, death of roots and at lower portion of shoot due to severe growth of several pathogenic and saprophytic seed associated fungi.

Table 1
Effect[†] of the polyvinylpyrrolidone (PVP) functionalized Zinc peroxide nanoparticles on spore germination of *Bipolaris sorokiniana in vitro* after 24 h of treatment

Concentration (ppm)	Spore germination (%)
1000	97.2 (79.77)b***3.0***
2000	96.0 (77.10)^{c}***3.7***
3000	95.8 (78.22)^{cb}***4.0***
4000	4.8 (12.61)^{d}***95.0***
5000	4.0 (8.87)^{d}***96.7***
6000	2.6 (9.61)^{e}***97.0***
7000	2.4 (8.87)^{e}***98.3***
8000	1.1 (5.88)^{f}***99.3***
9000	0.7 (3.70)^{g}***99.7***
10000	0.0 (0.0)^{h}***100.0***
Control[#]	100.0 (90.0) ^{a}***0.0***

[†]=Mean of five replicates; Figures in parentheses represent arcsine transformed values; Figures in bold italics represent % inhibition of spore germination after 24 hours; Means with the same letter(s) are not significantly different according to the complete randomized design at *P*=0.05

Table 2
Effect[†] of the polyvinylpyrrolidone (PVP) functionalized zinc peroxide (ZnO_2) nanoparticles on growth of *Bipolaris sorokiniana* causing foliar blight of wheat

Zinc peroxide (ZnO_2) Concentration (%)	Radial growth (mm) Days after inoculation			Mean
	2d	4d	6d	
0.5	10.2±0.44^{fg}	18.0±1.22^{ef}	24.2±1.30^{de}	17.0^{b}
1.0	9.4±0.54^{gh}	15.0±1.41^{f}	18.4±1.81^{e}	14.3^{c}
1.5	5.4±0.54^{h}	6.6±0.54^{h}	9.4±0.54^{g}	7.1^{d}
Control (0.0%)	26.8±0.44^{cd}	54.4±0.54^{cb}	84.8±2.86^{a}	55.3^{a}
Mean	13.0^{c}	23.5^{b}	34.2^{a}	

[†]=Mean of five replicates;±, Standard deviation; Means with the same letter(s) are not significantly different according to the factorial complete randomized design at *P*=0.05

Table 3
Effect† of the Polyvinylpyrrolidone (PVP) functionalized zinc peroxide (ZnO_2) nanoparticles spore production and spore size of *Bipolaris sorokiniana* causing foliar blight of wheat and wheat germination, its quality and mycoflora associated with wheat kernels

Zinc peroxide (ZnO_2) Concentration (%)	Seed germination (%)	Spore production ($100mm^2$) *	Spore measurement (µm)β			Seed germination remarks
			Length	Width	Size	
0.5	72(58.12)[a]	27705±308[b] (21.3)	17.8±2.3	6.1±1.0	17.8×6.1	• Fungi were unable to proliferate • Development of plume and radicle was with better length and greenness
1.0	68(55.58)[a]	18956±285[c] (57)	17.1±2.4	6.1±1.2	17.1×6.1	• Fungi were unable to proliferate • Development of plume and radicle was delayed and poor
1.5	48(46.15)[b]	0.0[d]	No spore production			• Fungi were unable to proliferate • Development of plume and radicle was delayed and poor
Control (0.0%)	52(43.84)[b]	35278±568[a]	22.6±1.5	9.2±2.8	22.6×9.2	• Several fungi, viz., *Bipolaris sorokiniana* , *Curvularia lunataAlternaria tenuis*, *A. alternata*, *Aspergillus flavus, Fusarium graminearum, Cladosporium spp. Penicillium spp.* were found associated with untreated seed

†=Mean of five replicates; ±, standard deviation; β, mean of 50 spores; Figures in parentheses represent arcsine transformed values; Figures in bold italics represent % inhibition of spore germination after 24 hours; Means with the same letter(s) are not significantly different according to the complete randomized design at P=0.05

Wani and Shah[50] carried out studies on the effect of MgO and ZnO nanoparticles on some plant pathogenic fungi viz. *Alternaria alternata, Fusarium oxysporum, Rhizopus stolonifer* and *Mucor plumbeus* in Kashmir stating that the effect of these nanoparticles on the inhibition of spore germination may be due to their fungicidal effect on the all the test fungi.

Conclusion

The proposed PVP functionalized ZnO_2 nanomaterial was as a kind of antifungal substance providing protection against fungi without its harmful impact on seed germination and can be used as a safer substitute of seed treating fungicides to protect environment and to reduce the cost of agricultural production. Moreover, it would not be exaggeration to state that ZnO_2 nanoparticles would be effective antifungal compounds capable of replacing fungicides harmful to environment and human health.

Further, the said material can also be tested against other seed borne diseases of various agriculturally important crops. The concentration can also be evaluated for proper use. The present studies open ways to modify the test ZnO_2 nanoparticles and to study against other plant diseases and phytotoxicity.

There are some questions still need to be addressed such as the exact mechanism of interaction of ZnO_2 nanoparticles with fungal cells and how the surface area of nanoparticles influences killing activity. Conversely, it is also interesting to know how the said material helps in promotion of plant growth and better seedling development. In addition to this, work is also required to know efficacy of the ZnO_2 nanoparticles against other seed borne diseases of important crops of national and international importance; standardization of doses, seed treatment methods and comparison with other nanoparticles. For its efficacy as antimicrobial agent at lower concentration from economy standpoint, the test material can be redesigned.

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