METHODS AND PROTOCOLS

Surface-modified sulfur nanoparticles: an effective antifungal agent against *Aspergillus niger* and *Fusarium oxysporum*

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Abstract Surface-modified sulfur nanoparticles (SNPs) of two different sizes were prepared via a modified liquid-phase precipitation method, using sodium polysulfide and ammonium polysulfide as starting material and polyethylene glycol-400 (PEG-400) as the surface stabilizing agent. Surface topology, size distribution, surface modification of SNPs with PEG-400, quantitative analysis for the presence of sulfur in nanoformulations, and thermal stability of SNPs were determined by atomic force microscopy (AFM), dynamic light scattering (DLS) plus high-resolution transmission electron microscopy (HR-TEM), fourier transform infrared (FT-IR) spectroscopy, energy dispersive X-ray (EDX) spectroscopy, and thermogravimetric analysis (TGA), respectively. A simultaneous study with microsized sulfur (S^0) and SNPs was carried out to evaluate their fungicidal efficacy against *Aspergillus niger* and *Fusarium oxysporum* in terms of radial growth, sporulation, ultrastructural modifications, and phospholipid content of the fungal strains using a modified poisoned food technique, spore-germination slide bioassay, environmental scanning electron microscopy (ESEM), and spectrometry. SNPs expressed promising inhibitory effect on fungal growth and sporulation and also significantly reduced phospholipid content.

Keywords Elemental sulfur · Sulfur nanoparticles · Antifungal · *Aspergillus niger* · *Fusarium oxysporum*

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Introduction

Elemental sulfur (S^0) is the most abundant multivalent inorganic non-metal on earth crust, which exists in several isotopic and allotropic forms. It is interesting to note that while sulfur plays a crucial role in protein synthesis machinery at a basal quantity, a high concentration of sulfur is however relatively toxic to microorganisms Madigan and Martinko (2005). This non-systemic and contact fungicide (Cooper and Williams (2004); Williams et al. 2002; Williams and Cooper 2004) is found to be effective against a large number of plant diseases like brown rot in peaches, powdery mildew diseases in apples, gooseberries, grapes, strawberries, sugar beets, etc., scab in roses as well as against certain smut and rust diseases of crop plants. A number of explanations have been put forward by researchers about the mode of action of S^0. First, S^0 depending upon its granule size, is taken up by pathogens and then oxidized to form fungitoxic pentathionic acid or reduced to form fungicidal hydrogen sulfide. H_2S promotes obstruction or oxidation of the free -SH attached forms of many essential enzymes within the cell and disrupts important intermediary metabolisms in mitochondria (Baldwin 1950; Libenson et al. 1953; McCallan 1949; Owens 1963). Second, fungicidal effect of S^0 also results from cross-linking of proteins or lipids with free

radical of sulfur. However, due to its high-volume requirement during application in agricultural fields and decelerated efficacy due to acquired resistance in target pathogens, use of S^0 as a fungicide is no more popular among farmers worldwide. Researchers also postulated that reduction in granule size would enhance the fungicidal efficacy of sulfur (Baldwin 1950). In recent studies, nano-form of S^0 as a fungicide (Roy Choudhury et al. 2010; Deshpande et al. 2008) was found to be more effective than the micron-sized S^0 owing to their increased surface/volume ratio and enhanced surface energy density (Halperin 1986; Nanda et al. 2003).

Aspergillus niger is an opportunistic, ubiquitous fungus and is a mycotoxigenic food and feed contaminant, especially of certain vegetables, fruits, nuts, beans, and cereals (Pfohl-Leszkowicz and Manderville 2007; Perrone et al. 2006; Perrone et al. 2007; Pitt and Hocking 1997). The fungus is also responsible for invasive aspergillosis in immunocompromised patients and significantly contributes to the increase of morbidity and mortality among neonates (Kanbe et al. 2002; Serrano et al. 2004). On the other hand, *Fusarium oxysporum* is a soil-borne plant pathogen, responsible for vascular wilts in a wide range of woods, crops, vegetables and ornamentals (Agrios 2005; Smith et al. 1988). Thiram, Zineb, Captan, Benomyl and Thiabendazole are widely used as fungicides in *A. niger*-affected crop fields (Kuthubutheen and Pugh 1979; Patterson et al. 2000; Serrano et al. 2004). Similarly, Carbendazim, Thiovit, Thiophanate-methyl and Benomyl are commonly used antifungal agents against *F. oxysporum* (Maraite and Meyer 1971; Rajput et al. 2006). Despite administration of these site-specific antifungal agents, effective control over these fungi remains unsatisfactory. Repeated use of synthetic pesticides also induces toxicity in the crop fields. Hence, development of alternative eco-safe antifungal agents like sulfur nanoparticles (SNPs) is urgently warranted. The present study deals with synthesis, characterization of two different-sized SNPs and their antifungal efficacy against *A. niger* and *F. oxysporum*. Antifungal property of SNPs was evaluated in terms of their effect on radial growth, frequency of spore formation, and phospholipid content of the fungal isolates.

Materials and methods

Chemicals

Sulfur powder (≥99.98% pure), sodium sulfide (≥98.0%, pure), ammonium sulfide (40–48 wt.% in H_2O), sodium hydroxide, ammonium hydroxide (28.0–30.0% NH_3 basis), sodium sulfate (≥99.0% pure), ammonium molybdate (99.98% pure), and ascorbic acid (≥99.0% pure, crystalline) were supplied by Sigma–Aldrich, MO, USA. Polyethylene

glycol-400, methanol, chloroform (GR), and sulfuric acid were supplied by Merck, Mumbai, India. Polyoxyethylene sorbitan mono-oleate (tween 80; >98% pure) and absolute ethanol were supplied by Fisher Scientific, Schwerte, Germany. Potato dextrose agar, saboraud dextrose agar and potato dextrose broth were supplied by Himedia laboratories Pvt. Ltd., Mumbai, India. Lactophenol-cotton blue was supplied by SRL Pvt. Ltd., Mumbai, India. Deionized water (18MΩ, arium 61316 reverse osmosis system, Sartorius Stedium Biotech, Aubagne, France) was used throughout the experiments.

Fungal strains and culture media

A. niger strains MTCC-282 and MTCC-2196 were purchased from Microbial Type Culture Collection, IMTECH, CSIR (Chandigarh, India). The wild strain (BDS-113) of *A. niger* was isolated from a rotten potato (obtained from commercial market in Calcutta, India) and was purified to single spore level, identified by phenotypic features, ITS/5.8 rRNA (HQ293217), and partial gene sequences for β-tubulin (HQ293218) and deposited in Microbial Type Culture Collection, India (MTCC accession number 10180). *F. oxysporum* strains NCIM-1072 and NCIM-1008 were purchased from National Collection of Industrial Microorganisms, NCL (Pune, India).

Synthesis of SNPs

SNPs were prepared by a modified liquid-phase precipitation method (Guo et al. 2005; Guo et al. 2006). Briefly, thoroughly pulverized sulfur powder (~3 μm) was mixed with sodium sulfide/ammonium sulfide (2 mol/l) solution and kept under continuous stirring until sodium polysulfide and ammonium polysulfide were formed. Approximately, 5 ml of polysulfide solution was then added to alkalinized (pH ≥9) PEG-400 solution (PEG-400: deionized H_2O in 5:1 ratio) and precipitated with aqueous formic acid (formic acid: deionized H_2O in 4:1 ratio). Excess PEG-400 was eliminated from SNPs surface with repeated alcohol washing followed by centrifugation. The resultant was finally subjected to a vacuum evaporator for the removal of residual alcohol.

Characterization of SNPs

The distributions of hydrodynamic diameters of SNPs were analyzed using dynamic light scattering (DLS) [Zetasizer nano series: Nano-S, Malvern, Worcestershire, UK] at various concentration, temperature, and pH. High-resolution transmission electron microscopy (HR-TEM) [201OF, JEOL Ltd., Tokyo, Japan] was carried out at 200 kV on a carbon-coated copper grid to confirm the

actual size of SNPs. Surface topology of the PEGylated SNPs was determined with atomic force microscopy (AFM) [di-CP-II with Proscan-19 software, Veeco, NY, USA]. Fourier transform infrared (FT-IR) [Shimazdu Corp., Kyoto, Japan] spectroscopy was done using potassium bromide (KBr) beads to ensure surface modification of SNPs with PEG-400. Purity and composition of SNPs was determined with energy dispersive X-ray (EDX) [FEI Quanta-200 MK-2, OR, USA] spectroscopy. Thermal stability of SNPs (range: ~50 to 500 °C in nitrogen) was determined with thermogravimetric analysis (TGA) [SII with Pyris manager software, Perkin Elmer, MA, USA].

Modified poisoned food technique

Radial growth of the fungal isolates was evaluated using a modified poisoned food technique (Dhingra and Sinclair 1995; Bhanumathi and Rai 2007; Pundir and Jain 2010). Briefly, spores were harvested with a sterile 0.8% tween-80 solution and the number of spores per milliliter was enumerated with a haemocytometer. Aliquots of a spore suspension at the concentration of ~10^4 spores/ml were used as inoculums. Antifungal media was prepared by mixing ethanol slurry of S^0 and SNPs with PDA at the concentration of 125, 500, 1,000, and 2,000 ppm for *A. niger* and at the concentration of 25, 50, 100, and 200 ppm for *F. oxysporum* strains. Ethanol containing agar plates were used as controls throughout the study. Five microliter of spore suspension (containing ~50 spores) was then inoculated in triplicate per plate and incubated for 48 h at 30 °C for *A. niger* strains. Inoculums of equal density were centrally spotted and incubated for 5 days at 28 °C in case of *F. oxysporum*. Radial growth (means of zone diameter) of the fungal isolates was measured manually followed by statistical analysis.

Spore-germination slide bioassay

Inhibitory effects of micron-sized S^0 and SNPs on sporulation of the fungal strains were determined with a slide bioassay (Resende et al. 1996; Wu et al. 2009). Briefly, fungal strains were cultured in potato dextrose broth at 28 °C and spores were harvested from 2 days (48 h) and 5 days (120 h) old cultures of *A. niger* and *F. oxysporum*, respectively. Approximately, 50 μl of spore suspension (~10^4 spores/ml) was stained with 25 μl of lactophenol-cotton blue and enumerated with haemocytometer under a light microscope (Zeiss Inc., NY, USA).

Environmental scanning electron microscopy (ESEM)

A comparative ESEM [FEI Quanta-200 MK-2, OR, USA] study with micron-sized S^0, and SNPs was performed to visualize their effect on ultrastructural modifications of hyphal structure in fungal isolates. ESEM samples were prepared under hydrated condition (Wojtas and Yang 1987), and images were obtained at 10-Kv vacuum.

Estimation of phospholipid content

One gram of mycelial mass from each of the fungi was used as starting material for determining moisture content before lipid and phospholipid analysis. Briefly, moisture content was determined by drying mycelial mass in an air oven at 110 °C until a constant weight was obtained (Firestone 1991). Total lipid extraction and estimation of phospholipid content were carried out following a standard method (Folch et al. 1957; Chen et al. 1956).

Statistical analysis

Reduction in radial growth between control and treated (with increasing concentrations of micron-size S^0 and SNPs) isolates for both the fungal species (considering all the strains) was evaluated with multiple-comparison analysis and two-way ANOVA employing MATLAB 7.6.0 (R2008a) software [The MathWorks Inc., MA, USA].

Results

Characterization of SNPs

DLS measurements revealed size distributions in hydrodynamic diameters in the range of 20–70 and 10–100 nm of SNPs prepared using sodium polysulfide (SNP-1) (Fig. 1a) and ammonium polysulfide (SNP-2) (Fig. 1c), respectively. HR-TEM micrographs revealed an average particle size ~20 and ~50 nm for SNP-1 (Fig. 1b) and SNP-2 (Fig. 1d), respectively. Average particle size of SNPs as revealed by HR-TEM were reasonably much lower than DLS measurements (size distributions in hydrodynamic diameters), as TEM can recognize the actual size of nanoparticles but not the surfactant layer due to their poor contrast. AFM studies showed that SNP-1(Fig. 2a; Table 1) differed from SNP-2 (Fig. 2b; Table 1) in terms of size, shape, and surface roughness. According to the EDX spectrum of SNP-1, 38.68 wt.% of sulfur is present in the sample (Fig. 3a). The EDX spectrum for SNP-2 revealed that 36.97 wt.% of sulfur is present in the sample (data not shown). The FT-IR spectrum of SNP-1 revealed two new absorption bands, which appeared at 671.18 and 1720.39 cm^{-1} when compared with that of PEG-400 (Fig. 3b). Similar results were found in case of SNP-2 (data not shown). The two bands were assigned to C-H bending vibrations and C=O stretching vibrations,
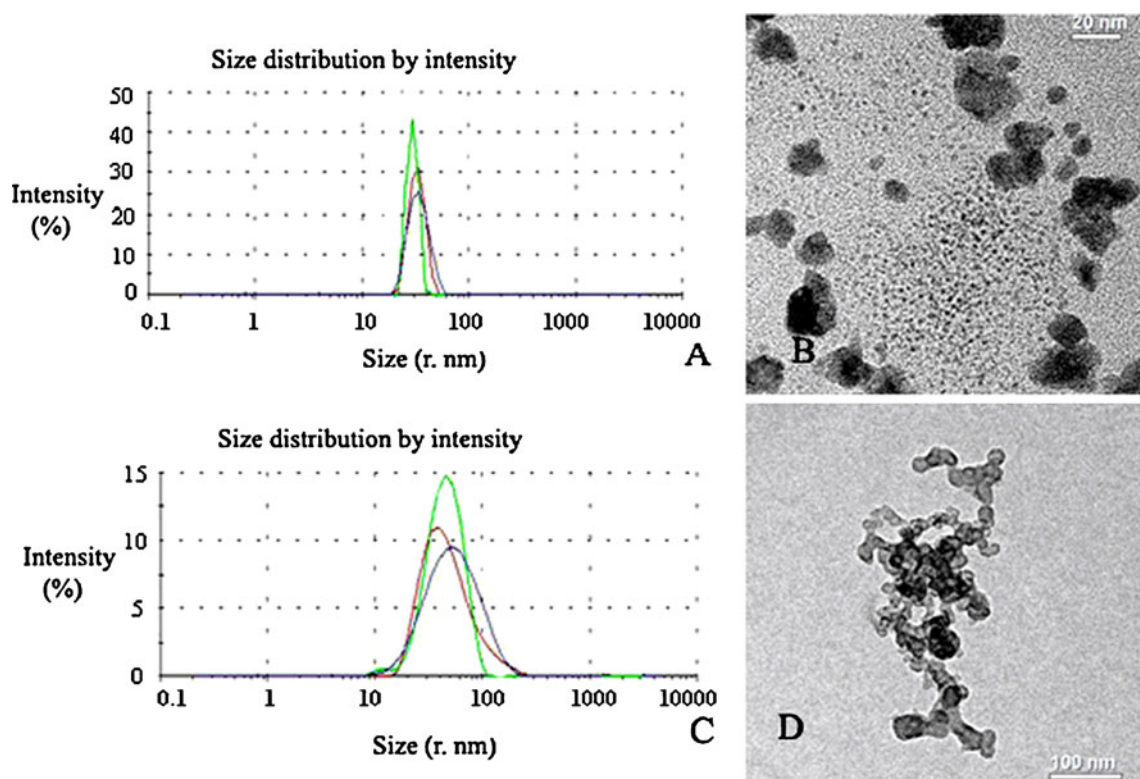
Fig. 1 Dynamic light scattering spectroscopy for size distribution of SNP-1 (**a**) and SNP-2 (**c**). High-resolution transmission electron micrographs for SNP-1 (**b**) and SNP-2 (**d**)

respectively (Moritz 1967; Nishio and Tsuchiya 2001). These vibrations seem to have been facilitated by the presence of SNP-1 in the composite. Furthermore, the stretching vibration band of C=C shifts by 3.86 cm^{-1} and

CH$_3$ bending vibration band shifts by 3.85 cm^{-1} (Sarapulova et al. 1998; Gerasimowicz et al. 1986) as compared to that of PEG (1,643.24 cm^{-1} and 1,456.16 cm^{-1}, respectively), indicating that PEG molecules coordinate at the surface of

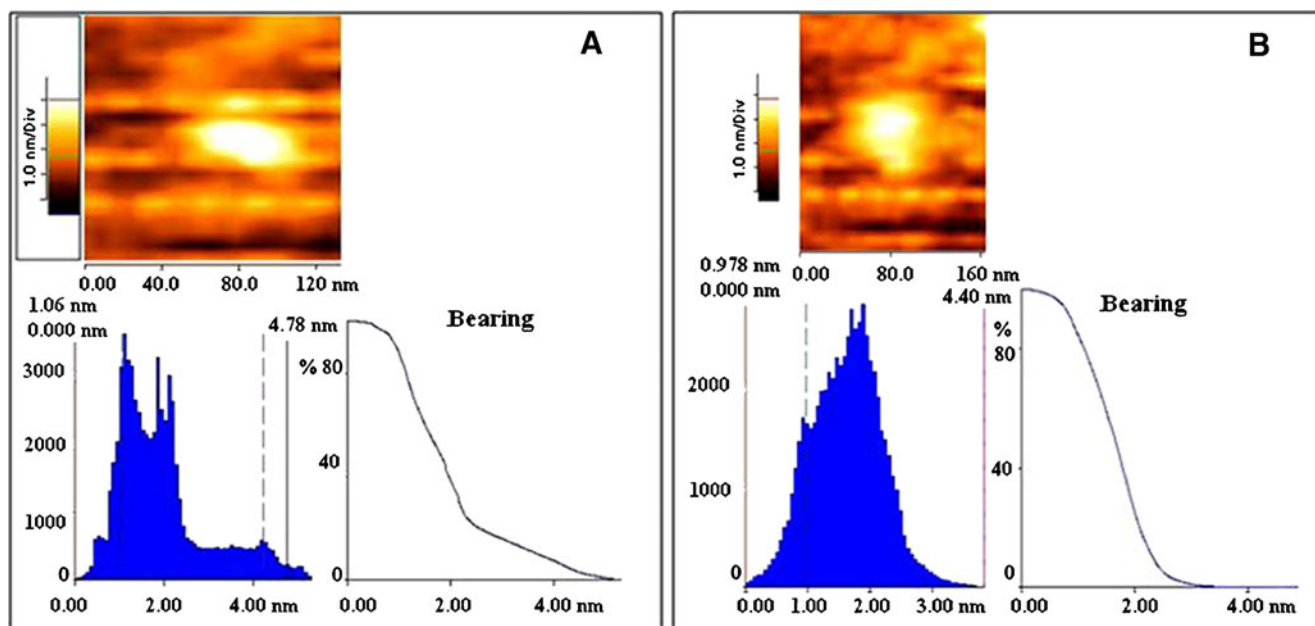**Fig. 2** Atomic force micrographs reveal surface topology of SNP-1 (**a**) and SNP-2 (**b**)

Table 1 Topological attributes of SNPs as determined from AFM studies

Type of SNPs	Rp-v (nm)	Rms rough (nm)	Average roughness (nm)	Mean height (nm)	Median height (nm)	Bearing ratio (nm)	Peak (nm)	Valley (nm)	Volume (μm³)	Surface area (μm²)	Projected area (μm²)
SNP-1 from sodium polysulfide	5.313	1.029	0.7827	1.961	1.756	@20.0%2.55	3.351	−1.961	$3.459e^{-005}$	0.01770	0.01764
SNP-2 from ammonium polysulfide	3.840	0.5631	0.4643	1.620	1.646	@20.0%2.09	2.220	−1.620	$4.142e^{-005}$	0.02573	0.02557

Rms Mean surface roughness; *Rp-v* Valley roughness

sulfur nanoparticles. The role of PEG is that of a template (Wu et al. 2008) with a possible van der Waals bond between PEG and SNPs. Thermogravimetric analyses (TGA) for both kinds of SNPs, as shown in Fig. 4a: SNP-1; b: SNP-2) were found to be non-isothermal. TGA studies also revealed that SNP-1 was more thermo-stable than that of SNP-2.

Modified poison food technique

Data shown in Fig. 5 represent the effects of SNP-1 and SNP-2 at a concentration of 125 ppm over ethanol (control) and micron-sized S⁰-treated strains (MTCC-282: panel A; MTCC-2196: panel B; MTCC-10180: panel C) of *A. niger*. Fig. 5d,e,f also statistically represents reduction in radial growth among fungal isolates, while treated with micron-sized and nanosized (SNP-1 and SNP-2) sulfur. At lower concentrations (25, 50, 75, and 100 ppm) SNPs expressed marginal efficacy in retarding radial growth of *A. niger* strains. However, a dose-dependent gradual reduction in radial growth was observed when all three *A. niger* strains were subjected to concentrations of 125 ppm and above of SNP-1 and SNP-2. In contrast, SNPs expressed an inhibitory effect against the tested *F. oxysporum* strains at the concentration as low as 25 ppm (Fig. 6). Unlike *A. niger* isolates, a gradual reduction in zone diameter with increasing concentration gradient of micron-sized S⁰ and SNPs was not obtained among *F. oxysporum* strains. However, for both the *F. oxysporum* strains (NCL-1008: Fig. 6a and NCL-1072: Fig. 6b) SNP-1 confers highest degree of reduction in zone diameter at the highest applied dose (200 ppm).

Fig. 3 Energy dispersive X-ray spectrum (**a**) and fourier transform-IR spectrum (**b**) for SNP-1, represents elemental composition and surface modification of the synthesized nanoparticles
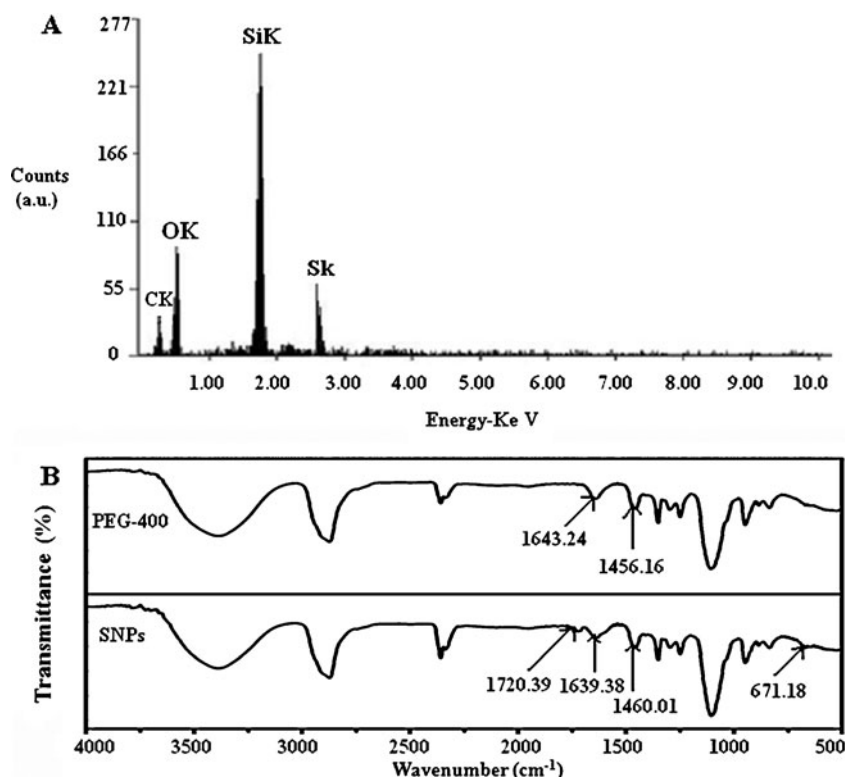
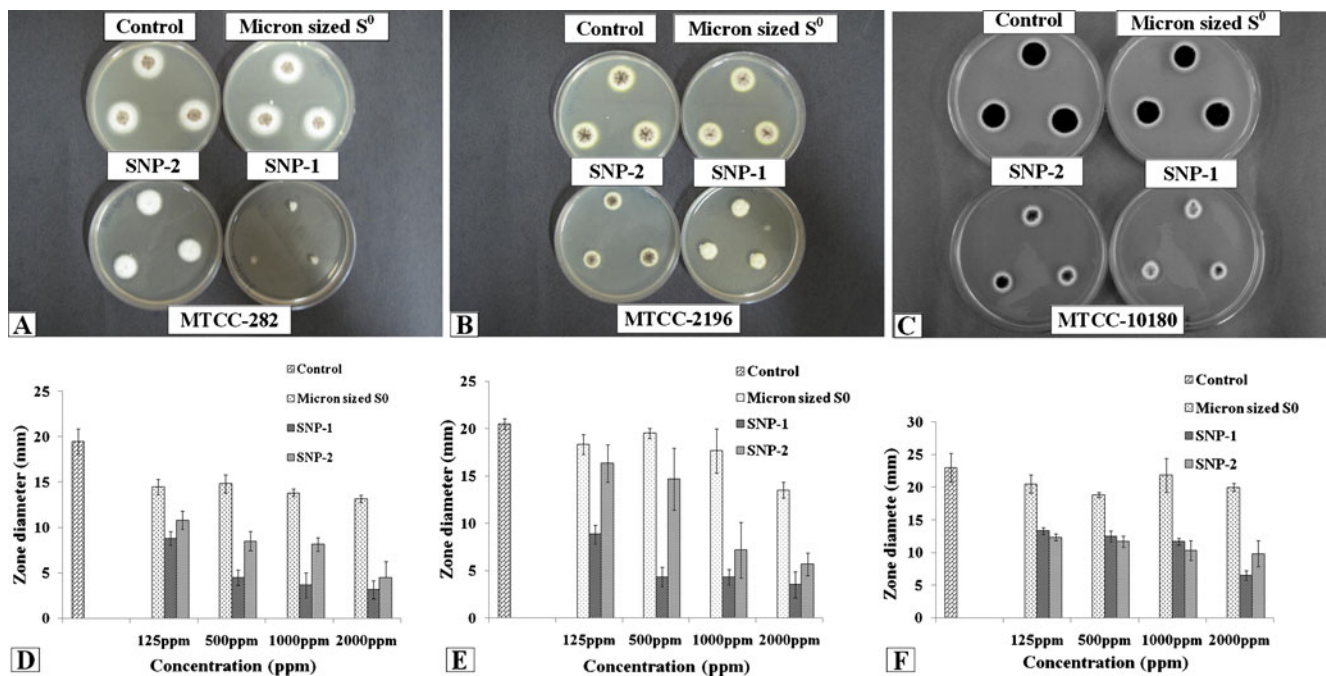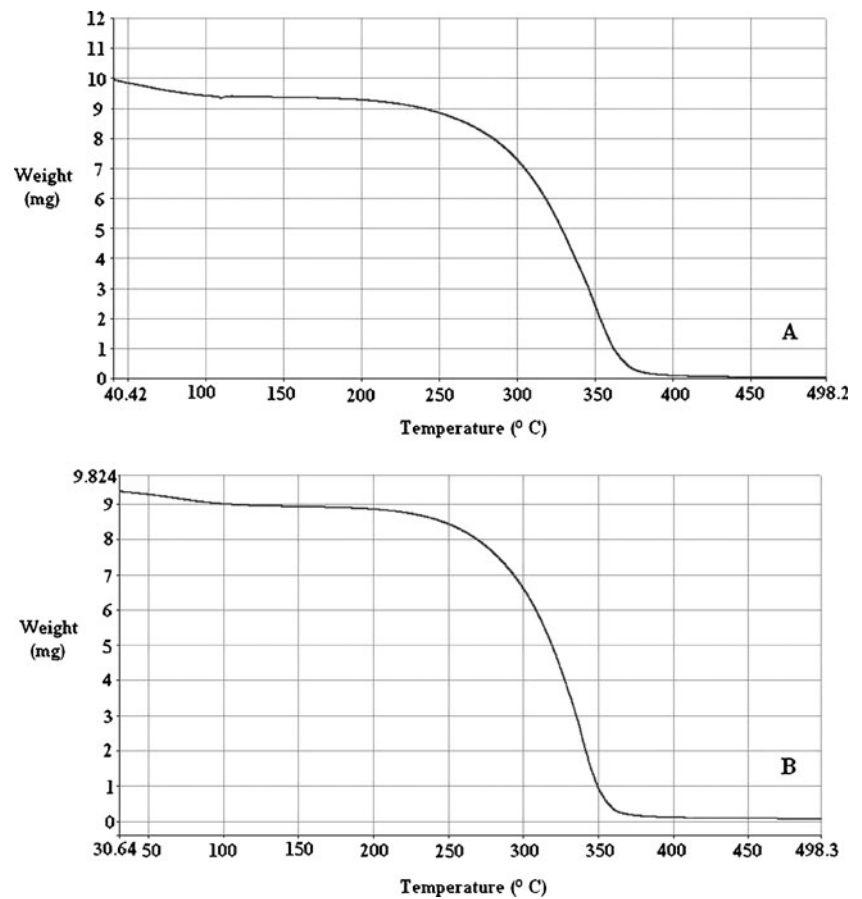Fig. 4 Thermal decomposition patterns of SNP-1 (**a**) and SNP-2 (**b**)

Fig. 5 Reduction in radial growth (means of zone diameter ± standard deviation) among *A. niger* strains; MTCC-282 (**a** and **d**), MTCC-2196 (**b** and **e**), and MTCC-10180 (**c** and **f**) after treatment with SNP-1, SNP-2, micron-sized S^0 and ethanol (control)
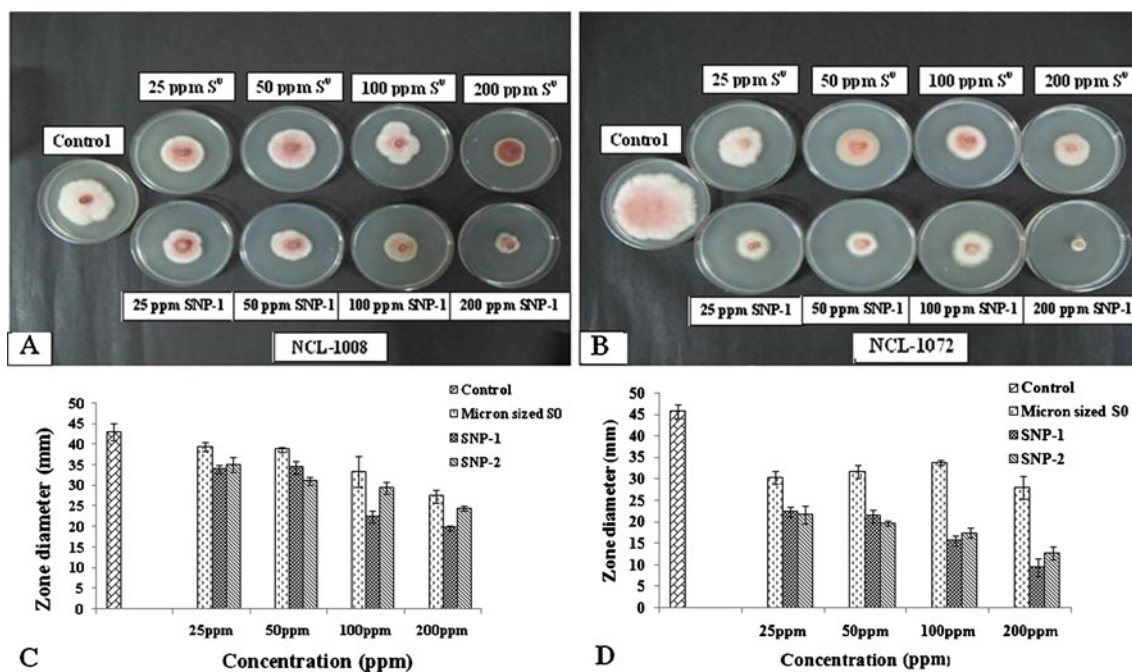
Fig. 6 Reduction in radial growth (means of zone diameter ± standard deviation) among *F. oxysporum* strains; NCL-1008 (**a** and **c**) and NCL-1072 (**b** and **d**) after treatment with SNP-1, SNP-2, micron-sized S^0 and ethanol (control)

Spore-germination slide bioassay

Means of germinating spores were enumerated (spores/ml) for 200 conidia at random for each concentration; of all the

A. niger and *F. oxysporum* strains (Table 2). Among micron-sized S^0-treated *A. niger* strains, no significant trend was found for reduction in spore germination. On the other hand, SNP-1 and SNP-2 not only reduce the rate

Table 2 Number of germinating spores among *A. niger* and *F. oxysporum* strains as obtained from a slide bioassay

Fungal strains: number of applied spores (spores/ml)	Concentration of S^0 and SNPs (ppm)	Number of germinated spores (spores/ml)		
		S^0	SNP-1	SNP-2
A. niger strain MTCC-10180 (BDS-113): 60×10^4	125	31×10^4	9×10^4	5×10^4
	500	33×10^4	6×10^4	3×10^4
	1,000	24×10^4	No spore found	1×10^4
	2,000	21×10^4	No spore found	No spore found
A. niger strain MTCC-282: 29×10^4	125	26×10^4	13×10^4	16×10^4
	500	17×10^4	No spore found	11×10^4
	1,000	19×10^4	No spore found	2×10^4
	2,000	17×10^4	No spore found	No spore found
A. niger strain MTCC-2196: 54×10^4	125	40×10^4	3×10^4	11×10^4
	500	32×10^4	6×10^4	10×10^4
	1,000	32×10^4	No spore found	2×10^4
	2,000	12×10^4	No spore found	2×10^4
F. oxysporum strain NCL-1008: 16×10^4	25	10×10^4	4×10^4	10×10^4
	50	6×10^4	7×10^4	8×10^4
	100	10×10^4	2×10^4	4×10^4
	200	4×10^4	No spore found	4×10^4
F. oxysporum strain NCL-1072: 15×10^4	25	12×10^4	9×10^4	7×10^4
	50	13×10^4	12×10^4	9×10^4
	100	8×10^4	6×10^4	4×10^4
	200	8×10^4	No spore found	No spore found

Fig. 7 Environmental scanning electron micrographs to visualize the effect of micron-sized S^0 and SNPs on hyphal morphology and conidiophores of *A. niger*. **a** and **b** effect of micron-sized S^0 and SNP-1 at 125 ppm concentration. **c** and **d**: effect of micron-sized S^0 and SNP-1 at a concentration of 2,000 ppm
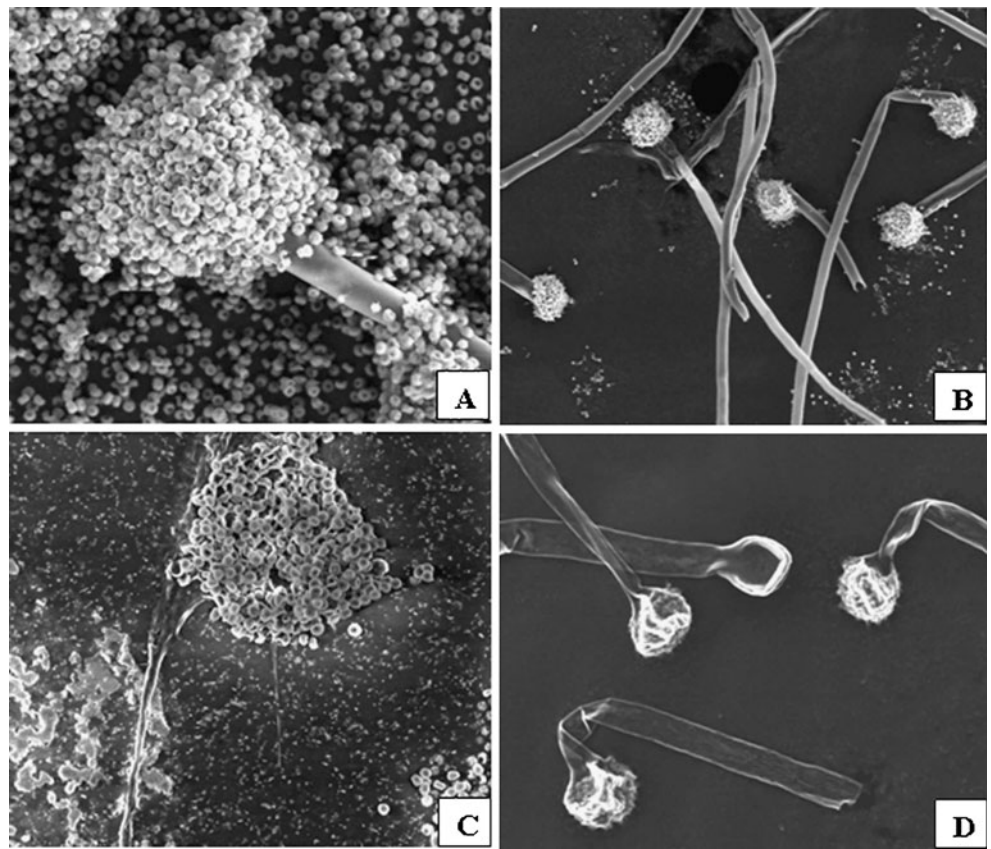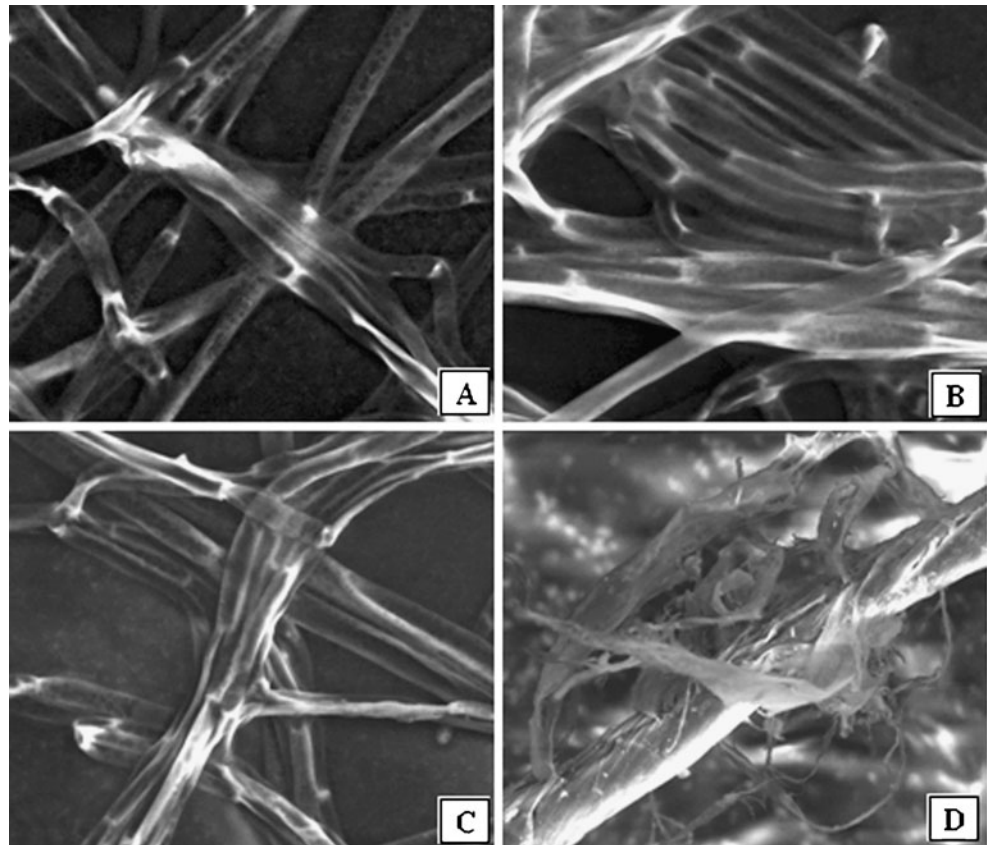

Fig. 8 Environmental scanning electron micrographs to visualize the effect of micron-sized S^0 and SNPs on hyphal morphology of *F. oxysporum*. **a** and **b**: effect of micron-sized S^0 and SNP-1 at 25 ppm concentration. **c** and **d**: effect of micron-sized S^0 and SNP-1 at a concentration of 200 ppm

of spore germination but also completely inhibit sporulation at different applied concentrations. In case of *F. oxysporum* strains, a general trend of spore germination retardation was observed for both micron-sized S[0] and SNPs, but a gradual dose dependency was not observed for either form of sulfur.

Statistical analysis

Statistical analysis has been made with the means of change in diameter of radial growth, obtained from three independent rounds of experiments. Statistical data analysis showed that there was significant reduction of fungal growth when SNP-1 and SNP-2 were used in comparison to that of control and S[0]. Two-way ANOVA and multiple-comparison analysis revealed that changes in zone diameter amongst SNP-1, SNP-2 and S[0] were statistically significant with p value of 0.014 for *A. niger*. In contrast, for *F. oxysporum*, it was found that the changes in zone diameter are not significantly different from each other (p value 0.3189). However, for both the fungal species, it can be concluded that SNP-1 showed better fungicidal effect than SNP-2 and micron-sized S[0].

ESEM study for fungal isolates

ESEM micrographs have been reported for the lowest effective and the highest applied dose-dependent effect of SNPs on ultrastructural modifications of hyphae and conidiophores. For *A. niger* strains, ESEM micrographs revealed signs of debilitation in hyphal morphology on treatment with 125 ppm concentration of SNP-1 (Fig. 7b). When 2,000 ppm of SNP-1 (Fig. 7d) and SNP-2 (data not shown) were used, shafts of conidiophores were heavily distorted and collapsed from their regular structure. In case of *F. oxysporum* strains, significant deformity in the hyphal structures was recorded only when the strains were subjected to the highest applied concentration (200 ppm) of SNPs. At 200 ppm concentration, SNPs were found to remove outer gelatinous layers (hyphal sheaths) from the surface of hyphae (Fig. 8d). It was also observed that the efficacy of SNP-1 (average particle size or APS ~20 nm, surface roughness 0.7827 nm) as a contact fungicide was better than SNP-2 (APS ~50 nm, surface roughness 0.4643 nm). This phenomenon can be correlated with the enhanced surface roughness and reduced size of SNP-1 compared to SNP-2.

Phospholipid content profile

The comparative ability of micron-sized S[0] and SNPs to perturb the hyphal membrane was assessed by measuring the phospholipid content of the mycelial biomass. Reduction in phospholipid content among micron-sized S[0] and SNPs-treated *A. niger* isolates (Fig. 9a) was found to be directly proportional to the increase in concentrations. On
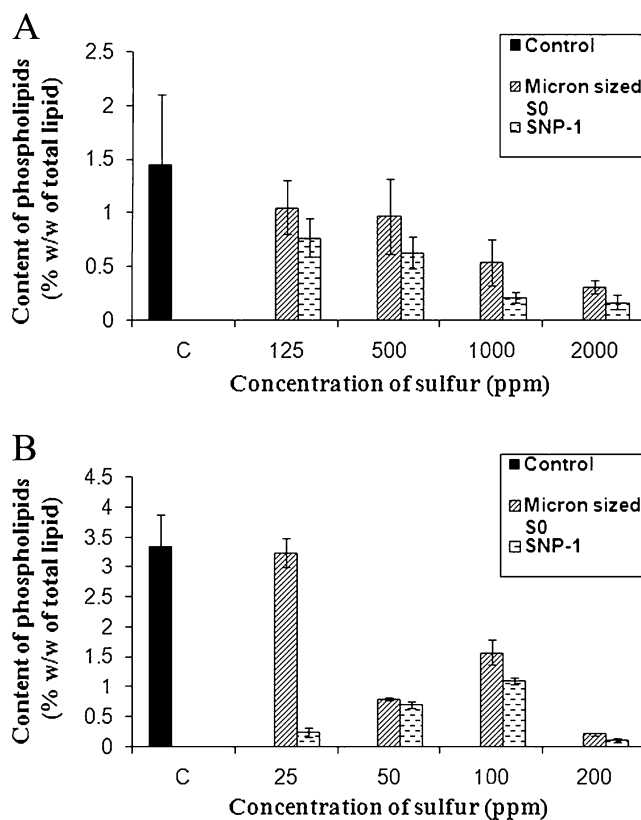

Fig. 9 Phospholipid content profile (% of *w/w* of total lipid ± standard deviation) of *A. niger* (**a**) and *F. oxysporum* (**b**) after treatment with micron-sized S[0], SNP-1 and ethanol (control)

the other hand, for *F. oxysporum*, a peculiar trend was observed in phospholipid content (Fig. 9b) while strains were treated with both micron-sized and nanoform of S[0]. A sudden surge in phospholipid content was found when the strains were treated with 100 ppm concentration of micron-sized S[0], while for SNPs, two upper consecutive concentrations (50 and 100 ppm) found to elevate phospholipid content in comparison to the lower concentration (25 ppm). Nonetheless, in both the fungal species, SNP-1 was found to reduce phospholipid content maximally at its highest applied dose.

Discussion

The efficacy of SNPs as a fungicide has been explored earlier (Roy Choudhury et al. 2010). In the present study, SNPs were synthesized with two different inorganic alkaline metal sulfides as starting materials, formic acid as a precipitating agent, and polyethylene glycol-400 (PEG-400) as a surface stabilizer. Formic acid (weak organic acid) was chosen in order to retain small size of SNPs. PEG-400 is a neutral ligand (with high HLB ratio) that hydrophilizes the surface and induces a steric barrier by anchoring long

and mobile PEG chains. Therefore, a PEGylated coat serves as protective agent (Torchilin 1998; Zalipsky 1995; Otsuka et al. 2003) on SNPs. Moreover, PEG-400 is a safe and biocompatible polymer that confers lesser toxicity than other surfactants and would be preferred by chemical industries. PEG coating on the surface of nanoparticles also makes them easily miscible in hydrophilic medium (agar/broth/other hydrophilic solvents). On the other hand, water insoluble non-capped micron-sized S^0 tends to settle down unevenly at the bottom of the hydrophilic medium (observation not shown). TGA analysis revealed that PEGylated SNP-1 and SNP-2 were stable even at 300 °C. This makes them suitable for use even in tropical crop fields where temperature may reach ~50 °C in summer. The results of the modified poison food technique with micron-sized S^0 and two types of SNPs validated that fungal growth inhibition is directly proportional to the increase in concentration and inversely proportional to the increase in particle size. The slide bioassay revealed no significant inhibitory trend for spore germination among micron-sized S^0-treated fungal isolates, but a degree of dose dependency was observed among all the strains of *A. niger* on treatment with SNPs. In *F. oxysporum* strains, the rate of sporulation was found to be independent of dose dependency. An anomalous rate of sporulation in *F. oxysporum* strains also suggests that at nanoscale dose dependency cannot be expected all the times. ESEM micrographs divulge that both kinds of PEGylated SNPs (SNP-1 and SNP-2) change hyphal morphology remarkably at the highest applied concentration, more efficiently than that of micron-sized S^0. At a concentration of 2,000 ppm, spherical heads of *A. niger* conidiophores are converted into hollow hemispheres. Significant changes in hyphal morphology were found in *F. oxysporum* when the fungus was treated with 200 ppm concentration of SNPs. All the above experiments strongly suggest that SNPs are better contact fungicides over micron-sized S^0 owing to their nanosized configuration, supramolecular surface reactivity, and potential transloca-tion capacity inside the fungal body. Reduction in radial growth and sporulation of *A. niger* strains MTCC-282 and MTCC-2196 were higher than the wild strain (MTCC-10180) when the strains were treated with SNPs. On the other hand, the NCL-1072 strain of *F. oxysporum* expressed a sudden surge in zone diameter while treated at the concentration of 100 ppm.

Due to resistance/semi-susceptibility to sulfur, *A. niger* isolates expressed a negligible or marginal reduction in zone diameter to the lower concentrations (25, 50, 75, and 100 ppm) of micron-sized S^0 and SNPs. In contrast, *F. oxysporum*, being a known sulfur susceptible fungus (Cooper and Williams 2004), was effectively controlled at a concentration as low as 25 ppm of SNPs. However, significant reduction was observed at and beyond the minimum effective concentration for both the fungal species while treated with SNP-1 and SNP-2. Treatment with SNPs considerably reduced fungal growth and rates of spore germination, and led to the logical demand for quantification of phosphorus in fungal bodies. Phos-pholipids in germinating spores serve as reservoirs of phosphorus during synthesis of nucleotides, sugars, and other morphogens involved in a number of developmental processes (Nishi 1961; Harold 1966; Lohmann et al. 1989). Change in phospholipid content as mentioned above, amongst *A. niger* and *F. oxysporum* strains insinuates that reduction in content of phospholipids is a highly species-specific phenomenon. However, it can be concluded that contact fungitoxicity of sulfur cannot be always correlated to its direct effect on phospholipid content of fungal body. Moreover, the effect of size specific SNPs on fungal morphogenesis warrants further study in this aspect. Promising fungicidal effects of other metal and metal oxide (e.g., Ag and ZnO) nanoparticles have already been reported elsewhere. However, enhanced phytotoxicity of the aforementioned particles (Lin and Xing 2007; Stampoulis et al. 2009) prohibits them from being applied in agro-medical sectors and logically demands the development of less toxic antifungal agents like SNPs. Therefore, all data taken together indicate that nano-sulfur would be a preferred alternative in agromedical sectors over existing chemical fungicides.

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