

# Chapter 6

## Synthesis and Applications of Nanofungicides: A Next-Generation Fungicide

Sonu Kumari and Suphiya Khan

**Abstract** With the increasing population, the pressure of enhancing food production and management of fungal diseases of food crops and fruits in agriculture sector needs urgent concern. Nanofungicides due to their vast physiochemical and functionalization properties could be easily applied for plant disease management. This chapter covers the different types of nanofungicide synthesis with mechanism. The chapter also gives comprehensive idea about fungal mycotoxins and its harmful effects on agricultural sector. Apart from it, this chapter also highlights the effects of nanoparticles (NPs) on mycotoxins produced by fungi and its mechanism of action.

### 6.1 Introduction

Nanoparticles (NPs) are defined as particles of size in range of 1–100 nm. Professor Norio Taniguchi of Tokyo Science University in 1974 first coined the term nanotechnology (Taniguchi 1974). The NPs nanosize provides great opportunities in several areas. Nanotechnology provides knowledge to synthesis nanostructures, and their unique properties show new application areas like environmental, pharmacology and medicine (Rai et al. 2009; Gupta et al. 2012; Aziz et al. 2015). In recent years, utilization of fungi as a source for NPs synthesis has been observed (Li et al. 2008).

NPs synthesis through different non-toxic and environment-friendly methods has been newly emerged. In this respect, there has been increasing research for NPs synthesis through microorganisms (Bernhardt et al. 2010). The NPs synthesis follows three steps that are appropriate solvent medium selection, reducing agent and stabilizing agents for NPs stabilization (Raveendran et al. 2003). Generally, this reduction mechanism is carried out by fungi enzymes for NPs synthesis (Kalimuthu et al. 2008; Kalishwaralal et al. 2008). Importantly, fungi have also secreted fairly

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large amount of proteins and secondary metabolites extracellularly, and hence, the fungal biomass could reduce the metal ions more easily leading to the rapid formation of NPs (Prasad et al. 2016). Because of these advances, the myco-based extracellular synthesis method is often considered as a better resource for higher productivity of NPs. The myconanoparticles play advantageous role over chemical antifungal agents as these have low tendency for microbial resistance. Among several inorganic NPs, silver has been comprehensively used because of its advantages over NPs such as copper, gold, zinc oxide NPs, etc. (Kalishwaralal et al. 2008).

Mycotoxins are secondary metabolites produced by fungi, and Bennett and Bentley (1989) defined it as “metabolic intermediates or products, found as a differentiation product in restricted taxonomic groups, not essential to growth and life of the producing organism, and biosynthesized from one or more general metabolites by a wider variety of pathways than is available in general metabolism”. Two groups were formed for toxigenic fungi infecting crops as “field” and “storage” fungi. Among all mycotoxins, aflatoxins, patulin, ochratoxin A, fumonisins and citrinin are considered the most disadvantageous types for crops. The confirmed mechanism for NPs action on fungi is not well understood. Many possible mechanisms were given by researchers, but the exact mechanism has not been reported.

This chapter provides insight into mycosynthesis of NPs and its mechanism. The different mycotoxins and its effects on crops were compiled along with NPs effects on the produced mycotoxins. The mechanism of action of NPs on fungi is also included.

## 6.2 Synthesis of Myconanoparticles

Nowadays, fungi are used as substrate for the production of different NPs. The fabrication of NPs using fungi has some practical advantages. Fungi have proved to be more beneficial for NPs synthesis compared to other microorganisms. Laboratory scale synthesis of fungi is done which provide the same biomass density as bacteria. Xu et al. (1999) and Taherzadeh et al. (2003) reported the biomass yield of  $0.31 \text{ g g}^{-1}$  and  $0.55 \text{ g g}^{-1}$  for *Escherichia coli* and *Rhizopus oryzae* culture when grown with glucose batch bioreactor. Several fungi species were reported for extracellular production of different NPs, such as *Penicillium*, *Fusarium oxysporum*, *Aspergillus* and *Verticillium* (Mukherjee et al. 2001; Gericke and Pinches 2006). Fungi can be used as excellent source of various extracellular enzymes which influence NPs synthesis (Saxena et al. 2014). There are several advantages of NPs synthesis from fungi over bacteria and plants.

### 6.2.1 Protein Secretion

Fungi secrete high concentration of extracellular enzymes that catalyze the heavy metal ions and form NPs. This results in faster production of NPs using fungi than chemical synthesis (Rai et al. 2009).

## 6.2.2 Isolation and Culture

Due to simple nutritional requirements, fungi are easy to isolate and subculture. There are various isolation methods for fungi such as plating, hyphal extraction and serial dilutions. Fungi are totipotent, and therefore hyphae or spores can be used to grow fungus and can be subcultured to obtain pure isolate (Rai et al. 2009).

## 6.2.3 Growth Control

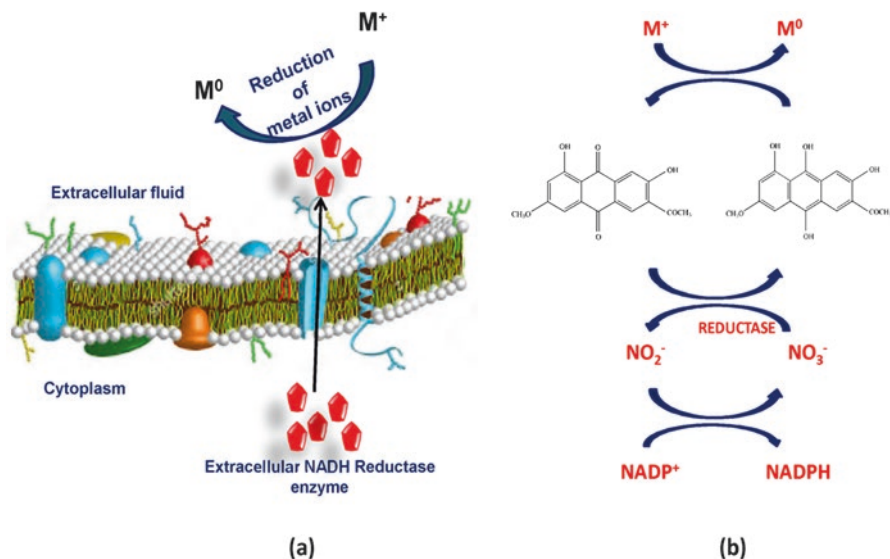
The several enzymes secreted by fungi can be used to synthesize NPs of defined size and shape. Fungi are able to maintain under high agitation and flow pressure as compared to bacteria and plants (Saha et al. 2010).

## 6.2.4 Extracellular Synthesis of NPs

Fungi can produce NPs extracellularly which is suitable for easier downstream processing and handling of biomass. Extracellular synthesis of silver NPs using *Aspergillus* sp. has been reported (Gade et al. 2008).

Fungi can produce NPs both intracellularly and extracellularly through two different mechanisms (Saxena et al. 2014).

Silver NPs were synthesized extracellularly using *Aspergillus fumigates* (Bhainsa and D'Souza 2006). The NPs synthesis mechanism is shown in Fig. 6.1. Gopinath and Arumugam (2014) investigated the synthesis of gold NPs from *F. solani* culture filtrate. TEM analysis showed the size of gold NPs was in the diameter range between 20 and 50 nm. Some researchers reported the biosynthesis of silver NPs by using *Trichoderma reesei*. In the production of silver NPs by *T. reesei*, mycelium contacts to the silver nitrate solution. This stimulates the fungus to secrete enzymes and metabolites for its survival mechanism. Further, the reduction of silver ions was done by secreted enzymes and metabolites. Chan and Don (2012) showed that *Schizophyllum commune* and *Pycnoporus sanguineus* could be used for biological synthesis of AgNPs. They used directly mycelia or culture supernatant of these white rot fungi for testing their reduction effect. The mycelia or culture supernatant produced AgNPs with different sizes (Chan and Don 2012). Cuevas et al. (2015) described the use of the extract of *Stereum hirsutum* for preparing copper and copper oxide NPs. In their study, three copper salts ( $\text{CuCl}_2$ ,  $\text{CuSO}_4$  and  $\text{Cu}(\text{NO}_3)_2$ ) were used, and the effect of various pH on reduction activity of this extract was investigated.  $\text{CuCl}_2$  (5 mM) gave the highest NPs formation at alkaline conditions. The extracellular protein in this extract may be responsible for NPs formation and stabilization. Sanghi and Verma (2009) described a continuous and extracellular formation of cadmium sulphide NPs (CdS) by immobilized *Coriolus versicolor* in a column reactor. The protein was reported as a capping agent. TEM analysis showed



**Fig. 6.1** Mechanism of extracellular NPs formation by fungi. (a) Extracellular NADH reductase enzyme action, (b) electron shuttle quinines action

the embedded NPs in the fungal matrix were well-dispersed spherical NPs with uniform size (about 5–9 nm) (Sanghi and Verma 2009). Bansal et al. (2005) showed that *F. oxysporum* secreted proteins competent of hydrolyzing zirconia ions at 30 °C. The research showed that metal halide precursors can be hydrolyzed by this fungus in acid medium (Bansal et al. 2005). To use as biosorbent agent, the group of SiO<sub>2</sub>-NPs (N-Si) with *P. funiculosum* for the synthesis of N-Si-Pen was investigated. This biosynthesized NP was utilized to adsorb Pb(II). The maximum capacity value was 1266.7 μmol g<sup>-1</sup> for N-Si-Pen combined particle. Sorption equilibrium was obtained in about 20 min.

Raliya and Tarafdar (2014) synthesized the magnesium, titanium and zinc NPs through the utilization of different fungus species, i.e., *A. niger*, *A. flavus*, *A. tubingenensis*, *A. fumigates*, *A. terreus* and *A. oryzae* with various precursor salts of chlorides, nitrates, oxides and sulphates (Raliya and Tarafdar 2014; Klaus et al. 1999). The various factors of protocol were also optimized for increasing the production of NPs. The biosynthesis of silver NPs by the cell-free filtrate of *P. nalgiovensis* was observed by the researcher (Maliszewska et al. 2014). The authors stated that proteins containing cysteine are responsible for reduction of metal ions for synthesis of NPs.

### 6.2.5 Intracellular NPs Synthesis

It was reported that NPs synthesized inside the organism could have smaller size as compared to extracellular NPs. Vigneshwaran et al. (2007) demonstrated the incubation for 72 h of *A. flavus* with silver nitrate for silver NPs synthesis. The NPs were

obtained on its cell wall surface with average particle size of 8.92 nm (Vigneshwaran et al. 2007). In addition, *Verticillium* sp. produced silver NPs on exposure with aqueous silver nitrate solution (Mukherjee et al. 2001). The *Verticillium* sp. was used for production of gold NPs, and NPs were detected on the surface and membrane of mycelium. Further, TEM analysis showed the hexagonal, spherical and triangular-shaped gold NPs on cell wall (Mukherjee et al. 2001). The gold NPs of less than 10 nm were synthesized using *V. luteoalbum* when incubated at pH 3.0 for 24 h (Gericke and Pinches 2006). Gold NPs were also synthesized intercellularly using *Trichothecium* sp. (Ahmad et al. 2005). The size of silver and gold NPs synthesized through fungi was in the range of 8.92–25 nm.

### 6.3 Mechanism of Mycosynthesis of NPs

The confirmed mechanism for NPs synthesis, for instance, of Ag NPs, in fungi is still not given, even though various researchers have worked to find possible mechanism for production of NPs (Duran et al. 2005; Meyer 2008). The mechanisms were reported for extracellular synthesis of NPs such as nitrate reductase action, electron shuttle quinones or both. The nitrate reductase method was observed due to reaction between nitrite and 2,3-diaminonaphthalene (Duran et al. 2005; Kumar et al. 2007). In many fungi species including *Penicillium*, the NPs synthesis was initiated by nitrate reductase, while some enzymes like extracellular shuttle quinone, alpha-NADPH-dependent reductases and nitrate-dependent reductases were responsible for silver NPs synthesis for *F. oxysporum*. The *A. flavus* was utilized for silver NPs production, which is possible due to “33 kDa” and cysteine protein which stabilizes the NPs by functioning as a capping agent (Jain et al. 2011). Most of the research paper favoured the nitrate reductase for NPs synthesis (Vigneshwaran et al. 2006; Perez-de-Luque et al. 2008; Deepa and Panda 2014). Fungal cell wall and cell wall sugars execute a major role for the absorption and reduction of metal ions (Sastry et al. 2003).

The proposed mechanisms by researchers for NPs intracellular synthesis consist of two steps (Kashyap et al. 2013):

- (a) The binding of metal ions on the fungal cell wall surface via electrostatic interaction between negatively charged carboxylate groups in enzymes of the mycelia cell wall and positive charge of metal ions.
- (b) Metals ions were reduced by enzymes, which lead to aggregation of NPs within the cell wall.

Extracellular NPs synthesis showed the interaction of metal ions and released enzymes (reductase), which leads to the formation of NPs in the solution (Kashyap et al. 2013). This method is advantageous than intracellular as its fungal cell lysis not required, NPs recovery and purification is easily achieved (Gade et al. 2008). The purification of NPs obtained through intracellular process is tedious task and requires time-consuming techniques. Kumar et al. (2007) showed the role of enzyme alpha-NADPH-dependent nitrate reductase in silver NPs synthesis.

## 6.4 Mycotoxins Produced by Fungi

Three genera of fungi were mainly reported for production of mycotoxins, namely, *Fusarium*, *Aspergillus* and *Penicillium* with dematiaceous fungal genera “*Alternaria*, *Helminthosporium*, *Drechslera*, *Phoma* and *Zygosporium*” (Ismail and Papenbrock 2014). The various fungi produced mycotoxins in plants during growing stage when environmental conditions are favourable. Toxigenic fungi in crops are grouped as:

- (a) Field fungi: fungi which form toxins before crop harvest. It depends on factors like plant host and environmental interactions (insects).
- (b) Storage fungi: these show problem after harvest in stored material. It depends on crop nutrients, moisture, temperature and insect's competition.

But the source of both field and storage fungi is field. Among the all known mycotoxins, aflatoxins, fumonisins, ochratoxin, citrinin and patulin are considered most harmful for food cereals. Aflatoxins are the major mycotoxins produced by genus *Aspergillus*. Almost 18 different aflatoxins were formed by *A. flavus* strains, and aflatoxin B1 is the extremely toxic and carcinogenic type (Ismail and Tharwat 2014; Richard et al. 2003). *Fusarium* sp. is responsible for the production of fumonisins (Thiel et al. 1991). Gelderblom et al. (1988) isolated the fumonisins B1 for the first time from *F. moniliforme* MRC 826. Ochratoxin was first discovered in 1965 in *A. ochraceus* (Van Der et al. 1965). Ochratoxin were mainly produced by *A. niger*, *A. carbonarius* and *P. verrucosum* fungal strains (Ciegler et al. 1977; Abarca et al. 1994; Horie 1995). Citrinin is another mycotoxin which was first obtained by Hetherington and Raistrick (1931) through *P. citrinum*. Several fungal species were identified for citrinin synthesis such as *P. verrucosum*, *M. ruber* and *A. terreus* (Ciegler et al. 1977). Chain et al. (1942) firstly isolated the patulin (PAT) mycotoxin from *P. claviforme* and named as calviformin, later renamed PAT due to production from *P. patulum*.

## 6.5 Phytotoxic Properties of Mycotoxins on Plant

Among all crop diseases, fungi are responsible for more than 70%, and fungi mycotoxins cause maximum crop loss in species such as cotton, rice, groundnut and wheat (Agrios 2005; Dhekney et al. 2007). Fungi inhibits the root parameters of plant more than shoot or mass causing various diseases such as browning, necrosis, lesions and wilt (McLean 1995; McLean et al. 1995). Several crop varieties were affected by mycotoxins produced by fungi such as cowpea, mung, sesame and gram. The carotenoid and chlorophyll synthesis and seed germination were also highly affected in some plants (Crisan 1973; Sinha and Kumari 1990; Sinha and Sinha 1993; Adekunle and Bassir 1997; Samuel and Valentine 2014). Inhibition of root and leaf development and chlorophyll synthesis was observed in some crop varieties due to aflatoxin (Reiss 1978). The lethal dose of aflatoxin was reported for

barley, sorghum and wheat as  $0.83 \text{ mg L}^{-1}$ ,  $2.75 \text{ mg L}^{-1}$  and  $1.74 \text{ mg L}^{-1}$ , respectively (Hasan 1999). Studies conducted showed the presence of necrosis and wilting when treated with concentration of  $1000 \text{ }\mu\text{g}\cdot\text{mL}^{-1}$  of fumonisins for soybeans (Abbas and Boyette 1992). Fumonisin severely affect the maize and tomato seedlings compared to aflatoxin mycotoxin (Lamprecht et al. 1994). The cell death in plants was obtained by the ochratoxin produced by fungi (Wang et al. 2011). The exposure of *A. thaliana* with ochratoxin caused growth inhibition and necrotic lesions (Peng et al. 2010). When *A. thaliana* plants leaves were treated with ochratoxin solutions, the macroscopic lesions were seen within 2 days (Wang et al. 2011). Ochratoxin showed inhibitory effect on *Zea mays* embryos with  $5 \text{ }\mu\text{g}\cdot\text{mL}^{-1}$  concentration (McLean et al. 1995). Citrinin showed phytotoxic effects in several trials conducted by researchers (White and Truelove 1972; Damodaran et al. 1975; Betina 1989; Maćias et al. 2000). Bean, sorghum and cotton plants demonstrated wilting effects when treated with citrinin (Damodaran et al. 1975). The seed number and flower number of crops were affected by patulin mycotoxin presence (Ellis and McCalla 1973; Ismaiel et al. 2014).

## 6.6 Effect of Nanoparticles on Fungi Mycotoxins

### 6.6.1 Aflatoxin

*Aspergillus flavus* is responsible for the synthesis of maximum number of aflatoxins. The chlorophyll synthesis in crops is mostly inhibited by it and responsible for low crop production. Cereals are mostly exposed to *A. flavus* attack and micromycete produced by them, so requirement for antifungal agents is developed (Al-othman et al. 2014). Nabawy et al. (2014) reported the antifungal potential of ZnO and  $\text{Fe}_2\text{O}_3$  NPs against isolated aflatoxigenic and non-aflatoxigenic *A. flavus* that were recovered from animal and poultry feeds associated with animal diseases using well and disc diffusion tests. The diameter zones of inhibition of non-aflatoxigenic strains were larger than in aflatoxigenic strains. The growth of all tested strains was not affected below the  $25 \text{ }\mu\text{g ml}^{-1}$  NPs concentration treatment. The well diffusion test proved better in studying of antifungal potential of NPs than disc diffusion. It is interesting to report here that the zone of *A. flavus* growth inhibition appeared at lower concentrations ( $50 \text{ }\mu\text{g ml}^{-1}$ ) of ZnO and  $\text{Fe}_2\text{O}_3$  NPs, whereas, similar effects in traditional antifungals required relatively higher concentration ( $100\text{--}200 \text{ }\mu\text{g ml}^{-1}$ ). Also, it was reported that the antifungal effects of clove oil against *A. flavus* showed comparatively lower antifungal effects than NPs in the study. Significant correlation between growth of *A. flavus* and aflatoxins production was clearly observed. The decreasing levels of aflatoxin B1 were reported with inhibition of fungal colonies till complete inhibition of both (Nabawy et al. 2014). Several studies evaluated the antimicrobial activity of NPs of metal oxide particularly ZnO powder against fungi in culture media. Regarding the results of antifungal activities of

ZnO, moulds as *A. flavus*, *A. niger* and *A. ochraceus* required higher concentration of ZnO NPs to inhibit their growth. The diameters of zones of inhibition of ZnO NPs against *A. flavus* and *A. ochraceus* were 7 and 15 mm at the concentration of 300  $\mu\text{g ml}^{-1}$ , whereas *A. niger* required relatively lower concentration (200  $\mu\text{g/ml}$ ) (6 mm) of NPs to inhibit its growth (Hassan et al. 2014). The antifungal activities of ZnO NPs against *P. expansum*, *Aspergillus* spp., *Rhizopus* spp. and yeast were investigated by other researchers (Violeta et al. 2011). The minimum inhibition concentration (MIC) of ZnO against *Aspergillus* spp. and *C. albicans* was reported to be 1.013–296  $\mu\text{g ml}^{-1}$  and for SDS and fluconazole was 0.001–0.56 and 0.062–128  $\mu\text{g ml}^{-1}$ , respectively. Moreover, it was added that the use of lower concentrations of ZnO NPs was the most effective antifungal and antibacterial. Furthermore, different studies conducted in different laboratories showed that the antimicrobial activity is influenced, not only by NPs concentration but also by the size of the ZnO particles (Violeta et al. 2011).

Silver NPs were also used in place of fungicides for reducing the growth and aflatoxin production by fungi. *A. terreus* was utilized for the synthesis of silver NPs, and experiment was conducted to analyse the effect of these on aflatoxin production by *A. flavus*. Inhibition in fungi growth and mycotoxin production was observed best at 150 ppm of NPs treatment. The fungi inhibition was seen through deformation of fungal hyphae and decrement in spore numbers (Al-othman et al. 2014).

### 6.6.2 Citrinin

Citrinin is mainly produced in crops after harvest and presents mainly in storage products such as fruits, grains, spices, beans, etc. (Da Lozzo et al. 2002; Bragulat et al. 2008). Magro et al. (2016) conducted a study for deletion of citrinin from *Monascus* suspensions. For this experiment, “surface active maghemite nanoparticles” (SAMNs) were synthesized, and 0.1  $\text{g L}^{-1}$  of *Monascus* suspension was treated with 1  $\text{g L}^{-1}$  of SAMNs. The 1  $\text{g L}^{-1}$  concentration of SAMNs removed 70% of citrinin and in second round with same concentration of NPs removed citrinin up to 0.2  $\text{mg L}^{-1}$ . This showed the binding of citrinin on SAMNs and formation of mycotoxin complex with iron (III). The formation of mycotoxin-SAMNs complex mainly depends on the presence of iron-chelating group on citrinin like keto-enol group (Magro et al. 2016).

### 6.6.3 Fumonisin

*Fusarium verticillioides*, *F. nygamai* and *F. proliferatum* are responsible for the production of fumonisins (Thiel et al. 1991; Rheeder et al. 2002). The decreased fungi growth and mycotoxin formation was achieved by zinc NPs. Mycotoxins synthesis level was lowered with increase in NPs concentration. The fumonisin B1



synthesizing fungus was inhibited by  $10 \mu\text{g ml}^{-1}$  zinc NPs treatment. SEM analysis revealed the rupture of fungi cell wall and reduction in the fumonisin production (Hassan et al. 2013).

#### 6.6.4 Ochratoxin

Mouhamed et al. (2015) evaluated the antifungal potential of ZnO and  $\text{Fe}_2\text{O}_3$  nanoparticles in comparison with some commercial antifungal feed additives (probiotic, propionic acid and clove oil) in inhibiting the growth of *Aspergillus ochraceus* and *Aspergillus niger* strains that were isolated from animal and poultry feeds using well and disc diffusion tests. The reported diameters of inhibition zones induced by metal NPs for non-ochratoxigenic strains were larger than that of ochratoxigenic strains, and the zone diameters increased when the NPs concentration increment. The  $20 \mu\text{g ml}^{-1}$  NPs concentration did not affect the growth of all *A. ochraceus* and *A. niger* strains, whereas the zones of inhibition produced by the metal NPs required lower concentration ( $25 \mu\text{g ml}^{-1}$  and more) than that produced by the commercial antifungal feed additives ( $50 \mu\text{g ml}^{-1}$  and more). The ochratoxin A production by ochratoxigenic strains in liquid medium or on yellow corn was significantly diminished in parallel with the decline parameters in colony count of the treated ochratoxigenic strains. The field application of the used NPs and other drugs on commercial animal feed evidenced the availability to use ZnO and  $\text{Fe}_2\text{O}_3$  NPs only as antifungal, but their antimycotoxin effect was limited to their use as feed additives during manufacture and before exposure of feeds to fungal contamination. Further studies were required for investigating the synergistic effects of combined antioxidant metal NPs and other commercial antimycotoxins to obtain dual synergistic actions in order to decrease the amount of used chemicals in the feed manufacture and to study the availability of its use in vivo (Mouhamed et al. 2015).

#### 6.6.5 Patulin

*Penicillium expansum* and *F. oxysporum* fungi are considered as the major source of a patulin and commonly found in rotting apples. Yehia and Ahmed (2013) conducted an experiment on *F. oxysporum* and *P. expansum* for analysing the toxic efficiency of NPs. *P. expansum* showed high inhibitory effect against ZnO NPs treatment than *F. oxysporum*. The mechanism of NPs action was explained as fungi growth inhibition was due to fungal hypha structure deformation. It was observed that patulin production was decreased by both fungi with the enhancement of NPs concentration. Two postharvest fungi, *Botrytis cinerea* and *P. expansum*, were treated with zinc oxide NPs with concentration of 3, 6 and  $12 \text{ mmol L}^{-1}$ . *P. expansum* growth was more inhibited by NPs activity (Yehia and Ahmed 2013).

## 6.7 Fungicidal Mechanisms of NPs

Das et al. (2009) conducted a study to assess the gold NPs effects on *S. cerevisiae* and *Candida albicans*. The study showed the mechanism of action of gold NPs on fungi. SEM analysis confirmed the rupturing of fungi cell wall due to NPs interaction and action (Das et al. 2009). The copper-based NPs interaction resulted in formation of reactive oxygen species (ROS) and caused DNA disruption (Chen et al. 2006; Oberdürster 2000; Heinlaan et al. 2008). Shah et al. (2010) reported the reduction in lignocellulose-degrading enzymes. In addition, the interaction with NPs also caused mitochondria and protein damage (Shah et al. 2010). The lethal effects of NPs are described in Fig. 6.2.

## 6.8 Advantages

NPs synthesis through fungi has proved advantageous compared to other organisms (Fig. 6.3). The fungi are favourable due to trouble-free isolation and capability of extracellular enzyme secretion (Singh et al. 2014; Prasad et al. 2016). Also, the process proved environmental friendly and less time-consuming for metal ion reduction by the secreted proteins of fungi (Rai et al. 2009).

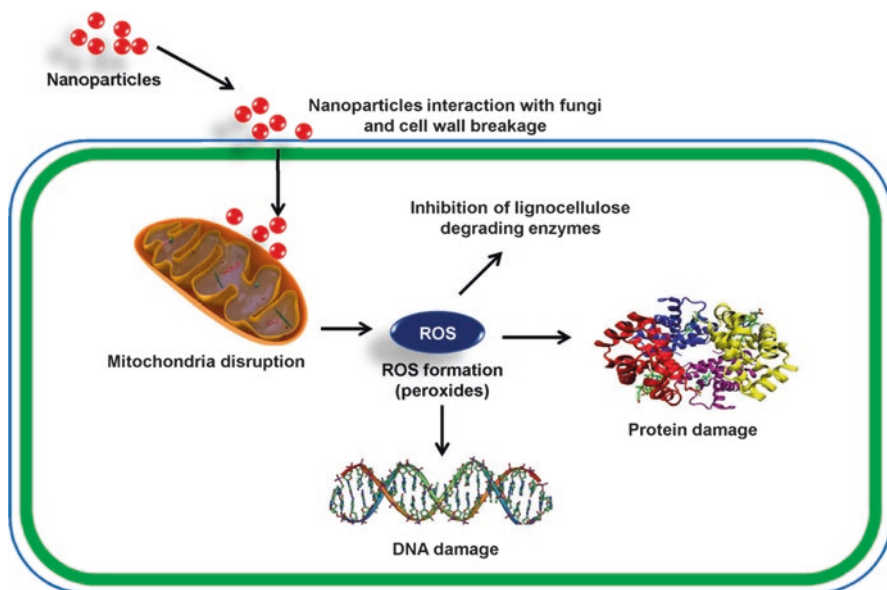
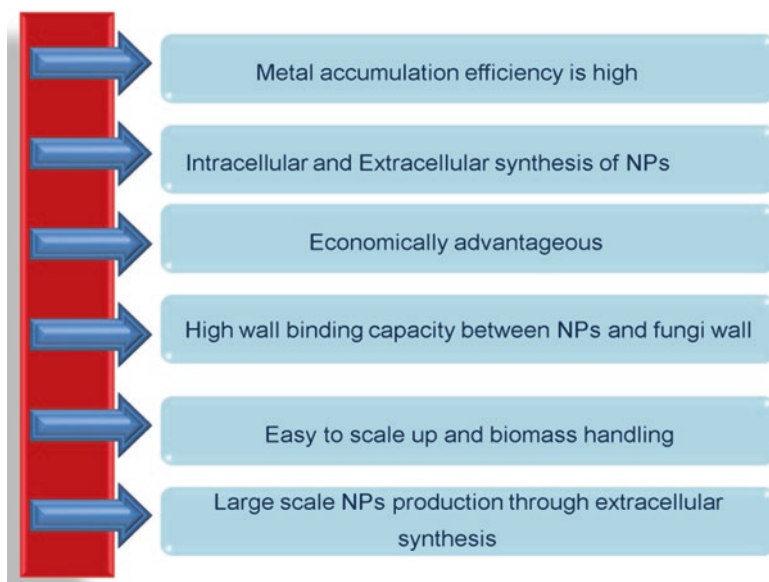


Fig. 6.2 Mechanism of antifungal action of NPs



**Fig. 6.3** Advantages of fungi for NPs synthesis

NPs are considered remarkable antifungal agents over chemical agents because of their low tendency to induce microbial resistance. The multiple modes of fungal inhibition by NPs also proved advantageous factor for using nanofungicides. The mentioned studies suggest that NPs can be used as an effective fungicide in agricultural and food safety applications (He et al. 2011; Bhattacharyya et al. 2016; Aziz et al. 2016; Ismail et al. 2017).

## 6.9 Conclusion

Nanotechnology is increasing in the agricultural sector for several applications. There is an increasing interest in NPs production through fungi due to its advantages over other sources. Several NPs were synthesized through fungi, but improvement in methods is required for controlled synthesis of NPs of required shape, composition and size. Mycotoxins were produced by several fungi, and effects on crops can be seen on basis of before harvest (field fungi) and after harvest (storage fungi). Aflatoxins, fumonisins, ochratoxin, citrinin and patulin were considered most harmful for crops produced by mainly *Aspergillus*, *Penicillium* and *Fusarium* species. The major disadvantage reported for agrochemicals used for various phytopathogenic fungi is the resistant developed pathogens. Therefore, NPs were explored as fungicides by several researchers. The studies suggested that the NPs are more effective fungicides as compared to agrochemicals used. The antimycotoxin effects

of NPs were limited to NPs dose provided. The antifungal property of NPs can be helpful in agricultural sector and food storage industries. It can be concluded that NPs are “new-generation fungicides” and can be synthesized by advantageous mycosynthesis process.

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## References

- Abarca ML, Bragulat MR, Castella G, Cabanes FJ (1994) Ochratoxin A production by strains of *Aspergillus niger* var. *niger*. *Appl Environ Microbiol* 60(7):2650–2652
- Abbas HK, Boyette CD (1992) Phytotoxicity of fumonisin B1 on weed and crop species. *Weed Technol* 1:548–552
- Adekunle AA, Bassir O (1997) The effects of aflatoxin B1 and G1 on the germination and leaf color of cowpea (*Vigna sinensis*). *Mycopathol Mycol Appl* 1997:1–2
- Agrios GN (2005) Plant pathology. Elsevier Academic Press, San Diego
- Ahmad A, Senapati S, Khan MI, Kumar R, Sastry M (2005) Extra-/intracellular biosynthesis of gold nanoparticles by an alkalotolerant fungus, *Trichothecium* sp. *J Biomed Nanotechnol* 1(1):47–53
- Al-Othman MR, ARM AE, Mahmoud MA, Fifan SA, El-Shikh MM (2014) Application of silver nanoparticles as antifungal and antiaflatoxin B1 produced by *Aspergillus flavus*. *Dig J Nanomater Bios* 1(9):151–157
- Aziz N, Faraz M, Pandey R, Shakir M, Fatma T, Varma A, Barman I, Prasad R (2015) Facile algae-derived route to biogenic silver nanoparticles: synthesis, antibacterial, and photocatalytic properties. *Langmuir* 31(42):11605–11612
- Aziz N, Pandey R, Barman I, Prasad R (2016) Leveraging the attributes of *Mucor hiemalis*-derived silver nanoparticles for a synergistic broad-spectrum antimicrobial platform. *Front Microbiol* 7:1984. doi:10.3389/fmicb.2016.01984
- Bansal V, Sanyal A, Rautaray D, Ahmad A, Sastry M (2005) Bioleaching of sand by the fungus *Fusarium oxysporum* as a means of producing extracellular silica nanoparticles. *Adv Mater* 17(7):889–892
- Bennett JW, Bentley R (1989) What’s in a name?-microbial secondary metabolism. *Adv Appl Microbiol* 34:1–28
- Bernhardt ES, Colman BP, Hochella MF, Cardinale BJ, Nisbet RM, Richardson CJ, Yin L (2010) An ecological perspective on nanomaterial impacts in the environment. *J Environ Qual* 39(6):1954–1965
- Betina V (1989) Mycotoxins. Chemical, biological and environmental aspects. Elsevier, Amsterdam
- Bhainsa KC, D’Souza SF (2006) Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigates*. *Colloids Surf B Biointerfaces* 47:160–164
- Bhattacharyya A, Duraisamy P, Govindarajan M, Buhroo AA, Prasad R (2016) Nano-biofungicides: emerging trend in insect pest control. In: Prasad R (ed) *Advances and applications through fungal nanobiotechnology*. Springer International Publishing, Cham, pp 307–319
- Bragulat MR, Martínez E, Castellá G, Cabañes FJ (2008) Ochratoxin A and citrinin producing species of the genus *Penicillium* from feedstuffs. *Int J Food Microbiol* 126(1):43–48

- Chain E, Florey HW, Jennings MA (1942) An antibacterial substance produced by *Penicillium claviforme*. Br J Exp Pathol 23(4):202
- Chan Y, Don MM (2012) Characterization of Ag nanoparticles produced by white-rot fungi and its in vitro antimicrobial activities. Int Arab J Antimicrob Agents 2(3):1–8
- Chen Z, Meng H, Xing G, Chen C, Zhao Y, Jia G, Wang T, Yuan H, Ye C, Zhao F, Chai Z (2006) Acute toxicological effects of copper nanoparticles in vivo. Toxicol Lett 163(2):109–120
- Ciegler A, Vesonder RF, Jackson LK (1977) Production and biological activity of patulin and citrinin from *Penicillium expansum*. Appl Environ Microbiol 33(4):1004–1006
- Crisan EV (1973) Effects of aflatoxin on seeding growth and ultrastructure in plants. Appl Microbiol 26(6):991–1000
- Cuevas R, Durán N, Diez MC, Tortella GR, Rubilar O (2015) Extracellular biosynthesis of copper and copper oxide nanoparticles by *Stereum hirsutum*, a native white-rot fungus from Chilean forests. J Nanomater 16(1):57
- Da Lozzo EJ, Mangrich AS, Rocha ME, de Oliveira MB, Carnieri EG (2002) Effects of citrinin on iron-redox cycle. Cell Biochem Funct 20(1):19–29
- Damodaran C, Kathirvel-Pandian S, Seeni S, Selvam R, Ganesan MG, Shanmugasundaram S (1975) Citrinin, a phytotoxin? Cell Mol Life Sci 31(12):1415–1417
- Das SK, Das AR, Guha AK (2009) Gold nanoparticles: microbial synthesis and application in water. Hyg Manag 25(14):8192–8199
- Deepa K, Panda T (2014) Synthesis of gold nanoparticles from different cellular fractions of *Fusarium oxysporum*. J Nanosci Nanotechnol 14(5):3455–3463
- Dhekney S, Li A, Anaman M, Dutt M, Tattersall J, Gray D (2007) Genetic transformation of embryogenic cultures and recovery of transgenic plants in *Vitis vinifera*, *Vitis rotundifolia* and *Vitis* hybrids. Acta Hort 738:743–748
- Durán N, Marcato PD, Alves OL, De Souza GI, Esposito E (2005) Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains. J Nanobiotechnol 3(1):8
- Ellis JR, McCalla TM (1973) Effects of patulin and method of application on growth stages of wheat. Appl Microbiol 25(4):562–566
- Gade AK, Bonde P, Ingle AP, Marcato PD, Duran N, Rai MK (2008) Exploitation of *Aspergillus niger* for synthesis of silver nanoparticles. J Biobased Mater Bio 2(3):243–247
- Gelderblom WC, Jaskiewicz K, Marasas WF, Thiel PG, Horak RM, Vleggaar R, Kriek NP (1988) Fumonisin – novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. Appl Environ Microbiol 54(7):1806–1811
- Gericke M, Pinches A (2006) Biological synthesis of metal nanoparticles. Hydrometallurgy 83(1):132–140
- Gopinath K, Arumugam A (2014) Extracellular mycosynthesis of gold nanoparticles using *Fusarium solani*. Appl Nanosci 4(6):657–662
- Gupta S, Sharma K, Sharma R (2012) Myconanotechnology and application of nanoparticles in biology. Recent Res Sci Technol 4(8):36–38
- Hasan HA (1999) Phytotoxicity of pathogenic fungi and their mycotoxins to cereal seedling viability. Mycopathologia 148(3):149–155
- Hassan AA, Howayda ME, Mahmoud HH (2013) Effect of zinc oxide nanoparticles on the growth of mycotoxigenic mould. SCEPT 1(4):66–74
- Hassan AA, Oraby NA, Mohamed AA, Mahmoud HH (2014) The possibility of using zinc oxide nanoparticles in controlling some fungal and bacterial strains isolated from buffaloes. Egypt J Appl Sci 29(3):58–83
- He L, Liu Y, Mustapha A, Lin M (2011) Antifungal activity of zinc oxide nanoparticles against *Botrytis cinerea* and *Penicillium expansum*. Microbiol Res 166(3):207–152
- Heinlaan M, Ivask A, Blinova I, Dubourguier HC, Kahru A (2008) Toxicity of nanosized and bulk ZnO, CuO and TiO<sub>2</sub> to bacteria *Vibrio fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus*. Chemosphere 71(7):1308–1316
- Hetherington AC, Raistrick H (1931) Studies in the Biochemistry of Micro-organisms. On the production and chemical constitution of a new yellow colouring matter, citrinin, produced from glucose by *Penicillium citrinum*. Trans Roy Soc (Lond) B 220:269–295

- Horie Y (1995) Productivity of ochratoxin a of *Aspergillus carbonarius* in *Aspergillus* section Nigri. Nippon Kingakukai Kaiho 36:73–76
- Ismaiel AA, Papenbrock J (2014) The effects of patulin from *Penicillium vulpinum* on seedling growth, root tip ultrastructure and glutathione content of maize. Eur J Plant Pathol 139(3):497–509
- Ismaiel AA, Papenbrock J (2014) The effects of patulin from *Penicillium vulpinum* on seedling growth, root tip ultrastructure and glutathione content of maize. Eur J Plant Pathol 139:497–509
- Ismail M, Prasad R, Ibrahim AIM, Ahmed ISA (2017) Modern prospects of nanotechnology in plant pathology. In: Prasad R, Kumar M, Kumar V (eds) Nanotechnology. Springer Nature Singapore Pte Ltd, Singapore, pp 305–317
- Jain N, Bhargava A, Majumdar S, Tarafdar JC, Panwar J (2011) Extracellular biosynthesis and characterization of silver nanoparticles using *Aspergillus flavus* NJP08: a mechanism perspective. Nanoscale 3(2):635–641
- Kalimuthu K, Babu RS, Venkataraman D, Bilal M, Gurunathan S (2008) Biosynthesis of silver nanocrystals by *Bacillus licheniformis*. Colloids Surf B Biointerfaces 65(1):150–153
- Kalishwaralal K, Deepak V, Ramkumarpandian S, Nellaiah H, Sangiliyadi G (2008) Extracellular biosynthesis of silver nanoparticles by the culture supernatant of *Bacillus licheniformis*. Mater Lett 62(29):4411–4413
- Kashyap PL, Kumar S, Srivastava AK, Sharma AK (2013) Myconanotechnology in agriculture: a perspective. World J Microbiol Biotechnol 29(2):191–207
- Klaus T, Joerger R, Olsson E, Granqvist CG (1999) Silver-based crystalline nanoparticles, microbially fabricated. Proc Natl Acad Sci 96(24):13611–13614
- Kumar SA, Abyaneh MK, Gosavi SW, Kulkarni SK, Pasricha R, Ahmad A, Khan MI (2007) Nitrate reductase-mediated synthesis of silver nanoparticles from AgNO<sub>3</sub>. Biotechnol Lett 29(3):439–445
- Lamprecht SC, Marasas WF, Alberts JF, Cawood ME, Gelderblom WC, Shephard GS, Thiel PG, Calitz FJ (1994) Phytotoxicity of fumonisins and TA-toxin to corn and tomato. Phytopathology 84(4):383–391
- Li Q, Mahendra S, Lyon DY, Brunet L, Liga MV, Li D, Alvarez PJ (2008) Antimicrobial nanomaterials for water disinfection and microbial control: potential applications and implications. Water Res 42(18):4591–4602
- Macías M, Ulloa M, Gamboa A, Mata R (2000) Phytotoxic compounds from the new coprophilous fungus *Guanomyces polythrinx*. J Nat Prod 63(6):757–761
- Magro M, Moritz DE, Bonaiuto E, Baratella D, Terzo M, Jakubec P, Malina O, Čépe K, de Arago GM, Zboril R, Vianello F (2016) Citrinin mycotoxin recognition and removal by naked magnetic nanoparticles. Food Chem 203:505–512
- Maliszewska I, Juraszek A, Bielska K (2014) Green synthesis and characterization of silver nanoparticles using ascomycota fungi *Penicillium nalgiovense* AJ12. J Clust Sci 25(4):989–1004
- McLean M (1995) The phytotoxicity of selected mycotoxins on mature, germinating *Zea mays* embryos. Mycopathologia 132(3):173–183
- McLean M, Watt MP, Berjak P, Dutton MF (1995) Aflatoxin B1-its effects on an in vitro plant system. Food Addit Contam 12(3):435–443
- Meyer V (2008) Genetic engineering of filamentous fungi-progress, obstacles and future trends. Biotechnol Adv 26(2):177–185
- Mouhamed AE, Hassan AA, Manal AH, El Hariri M, Refai M (2015) Effect of metal nanoparticles on the growth of Ochratoxigenic moulds and Ochratoxin A production isolated from food and feed. Int J Res Stud Biosci 3(9):1–14
- Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar SR, Khan MI, Parishcha R, Ajaykumar PV, Alam M, Kumar R, Sastry M (2001) Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: a novel biological approach to nanoparticle synthesis. Nano Lett 1(10):515–519
- Nabawy GA, Hassan AA, Sayed El-Ahl RH, Refai MK (2014) Effect of metal nanoparticles in comparison with commercial antifungal feed additives on the growth of *Aspergillus flavus* and aflatoxin b1 production. J Glob Biosci 3:954–971

- Oberdürster G (2000) Toxicology of ultrafine particles: in vivo studies. *Philos Trans R Soc Lond A: Math Phys Eng Sci* 358(1775):2719–2740
- Peng XL, Xu WT, Wang Y, Huang KL, Liang ZH, Zhao WW, Luo YB (2010) Mycotoxin ochratoxin A-induced cell death and changes in oxidative metabolism of *Arabidopsis thaliana*. *Plant Cell Rep* 29(2):153–161
- Perez-de-Luque A, Rubiales D, Marquina CI, Ibarra MR, De la Fuente JM (2008) Nanoparticles in agriculture, development of smart delivery systems for plant research. *Nano-Spain, Braga*
- Prasad R, Pandey R, Barman I (2016) Engineering tailored nanoparticles with microbes: quo vadis? *Wiley Interdiscip Rev Nanomed Nanobiotechnol* 8(2):316–330
- Rai M, Yadav A, Bridge P, Gade A, Rai MK, Bridge PD (2009) Myconanotechnology: a new and emerging science. *Appl Mycol* 14:258–2567
- Raliya R, Tarafdar JC (2014) Biosynthesis and characterization of zinc, magnesium and titanium nanoparticles: an eco-friendly approach. *Int Nano Lett* 4(1):1–0
- Raveendran P, Fu J, Wallen SL (2003) Completely green synthesis and stabilization of metal nanoparticles. *J Am Chem Soc* 125(46):13940–13941
- Reiss J (1978) Effects of mycotoxins on higher plants, algae, fungi and bacteria. *Mycotoxic Fungi Mycotoxins Mycotoxicoses* 3:118–144
- Rheeder JP, Marasas WF, Vismer HF (2002) Production of fumonisin analogs by *Fusarium* species. *Appl Environ Microbiol* 68(5):2101–2105
- Richard JL, Payne GA, Desjardin AE, Maragos C, Norred WP, Pestka JJ (2003) Mycotoxins, risks in plant, animal and human systems. In: CAST Task Force Report 139; Council for Agricultural Science and Technology: Ames, 101–103. 32
- Saha S, Sarkar J, Chattopadhyay D, Patra S, Chakraborty A, Acharya K (2010) Production of silver nanoparticles by a phytopathogenic fungus *Bipolaris nodulosa* and its antimicrobial activity. *Dig J Nanomater Biostruct* 5(4):887–895
- Samuel AT, Valentine IT (2014) Effect of total aflatoxin on the growth characteristics and chlorophyll level of sesame (*Sesamum indicum* L.) *NY Sci J* 7:8–13
- Sanghi R, Verma P (2009) A facile green extracellular biosynthesis of CdS nanoparticles by immobilized fungus. *Chem Eng J* 155(3):886–891
- Sastry M, Ahmad A, Khan MI, Kumar R (2003) Biosynthesis of metal nanoparticles using fungi and actinomycete. *Curr Sci* 85(2):162–170
- Saxena J, Sharma MM, Gupta S, Singh A (2014) Emerging role of fungi in nanoparticle synthesis and their applications. *World J Pharm Sci* 3:1586–1613
- Shah V, Dobiášová P, Baldrian P, Nerud F, Kumar A, Seal S (2010) Influence of iron and copper nanoparticle powder on the production of lignocellulose degrading enzymes in the fungus *Trametes versicolor*. *J Hazard Mater* 178(1):1141–1145
- Singh D, Rathod V, Ninganagouda S, Hiremath J, Singh AK, Mathew J (2014) Optimization and characterization of silver nanoparticle by endophytic fungi *Penicillium* sp. isolated from *Curcuma longa* (turmeric) and application studies against MDR *E. coli* and *S. aureus*. *Bioinorg Chem Appl* 2014:1–8
- Sinha KK, Kumari P (1990) Some physiological abnormalities induced by aflatoxin B1 in mung seeds (*Vigna radiata* variety *Pusa Baishakhi*). *Mycopathologia* 110(2):77–79
- Sinha KK, Sinha AK (1993) Effect of aflatoxin B1 on germination index and seedling growth in wheat varieties. *Mycopathologia* 123(3):165–169
- Taherzadeh MJ, Fox M, Hjorth H, Edebo L (2003) Production of mycelium biomass and ethanol from paper pulp sulfite liquor by *Rhizopus oryzae*. *Bioresour Technol* 88(3):167–177
- Taniguchi N (1974) On the basic concept of nanotechnology. In: Proceedings of the international conference on production engineering, Tokyo, Part II, Japan Society of Precision Engineering, pp 18–23
- Thiel PG, Marasas WF, Sydenham EW, Shephard GS, Gelderblom WC, Nieuwenhuis JJ (1991) Survey of fumonisin production by *Fusarium* species. *Appl Environ Microbiol* 57(4):1089–1093
- Van der Merwe KJ, Steyn PS, Fourie L, Scott DB, Theron JJ (1965) Ochratoxin A, a toxic metabolite produced by *Aspergillus ochraceus* Wilh. *Nature* 205(4976):1112–1113

- Vigneshwaran N, Ashtaputre NM, Varadarajan PV, Nachane RP, Paralikar KM, Balasubramanya RH (2006) Biological synthesis of silver nanoparticles using the fungus *Aspergillus flavus*. *Mater Lett* 61:1413–1418
- Vigneshwaran N, Ashtaputre NM, Varadarajan PV, Nachane RP, Paralikar KM, Balasubramanya RH (2007) Biological synthesis of silver nanoparticles using the fungus *Aspergillus flavus*. *Mater Lett* 61(6):1413–1418
- Violeta V, Catalin P, Constantin F, Monica A, Marius B (2011) Nanoparticles applications for improving the food safety and food processing. In: 7th international conference on materials science and engineering, Bramat, Brasov, vol 77, 24–26 Feb 2011
- Wang Y, Peng X, Xu W, Luo Y, Zhao W, Hao J, Liang Z, Zhang Y, Huang K (2011) Transcript and protein profiling analysis of OTA-induced cell death reveals the regulation of the toxicity response process in *Arabidopsis thaliana*. *J Exp Bot* 29:err447
- White AG, Truelove B (1972) The effects of aflatoxin B1, citrinin, and ochratoxin A on amino acid uptake and incorporation by cucumber. *Can J Bot* 50(12):2659–2664
- Xu B, Jahic M, Blomsten G, Enfors SO (1999) Glucose overflow metabolism and mixed-acid fermentation in aerobic large-scale fed-batch processes with *Escherichia coli*. *Appl Microbiol Biotechnol* 51(5):564–571
- Yehia RS, Ahmed OF (2013) In vitro study of the antifungal efficacy of zinc oxide nanoparticles against *Fusarium oxysporum* and *Penicillium expansum*. *Afr J Microbiol Res* 7(19):1917–1923