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REVIEWS

Photocatalytically Active Zinc Oxide and Titanium Dioxide Nanoparticles in Clonal Micropropagation of Plants: Prospects

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Abstract—The search for effective and nontoxic sterilization drugs for plants against common phytopathogenic microorganisms is a major challenge to improve the biotechnology of plant clonal micropropagation. An analysis of 92 studies that describe the potential use of ZnO and TiO_2 nanoparticles as antimicrobial agents in biotechnology showed that their biological effects depend on several factors: photocatalytic activity, particle size, concentration, morphology, and surface modification. The mechanisms of toxicity, among which the primary one is generation of reactive oxygen species leading to oxidative stress, are also due to these factors. The data describing the direct influence of ZnO and TiO_2 nanoparticles on plants, however, are contradictory, which is probably because of the various particle shapes and sizes, their concentrations, and the species characteristics of the plants studied. These studies have confirmed that photocatalytically active ZnO and TiO_2 nanoparticles may be used as bactericidal and fungicidal drugs for sterilization of explants during clonal micropropagation of plants, while taking into account the possible phytotoxicity of these particles.

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INTRODUCTION

One of the main problems that arise during preparation of planting material is phytopathologies caused by various microorganisms: fungi and, less often, bacteria together with viruses. Microbial contamination is a serious threat, especially for plant tissue culture, because it can destroy explants. The organs obtained from plants under field conditions or in a greenhouse have previously undergone surface sterilization prior to introduce into the culture. The disinfection of explants is an important step before in vitro cultivation, because microorganisms in the growth medium grow faster than explants and can seriously affect the results of microclonation. Sterilization, however, often seems to be rather ineffective. Disinfectants (bromine water, calcium hypochlorite, ethanol, hydrogen peroxide, sodium hypochlorite, mercuric chloride, silver nitrate, antibiotics, and fungicides) are conventionally used to obtain sterile explants, but an increase in the concentration of disinfectants and the time required for sterilization negatively affect the quality and viability of explants. In addition, some substances are phytotoxic [1, 2].

Nanoparticles and nanomaterials are currently considered to be "new antibiotics." In particular, zinc oxide and titanium dioxide nanoparticles [3, 4] (Figs. 1, 2) are promising antimicrobial agents, because they possess photocatalytic activity, high penetration, and relative safety for multicellular organisms, at least in comparison with many other means for sterilization of explants.

It should be noted that nanoparticles may also be used in the preparation of various sensors, herbicides, phytoimmunity stimulants, and agents for pesticide removal from plants and soil, in addition to the development of nanopesticides (Fig. 3) [5].

Antibacterial Properties of ZnO and TiO_2 Nanoparticles

Nanosized zinc oxide can possess high antibacterial activity against various bacteria and fungi, and a significant number of works confirms this fact [6–13]. The bactericidal properties of zinc oxide are currently being studied in both the macro- and nanoforms. The authors have shown that ZnO has greater antimicrobial activity when the particle size decreases to the nanometer range, because ZnO nanoparticles can interact with the cell surface and/or nucleus during penetration into the cell [9].

Although the biocidal activity of ZnO has been studied quite well, the exact mechanism of toxicity is not fully explained and is controversial. The main possible mechanism discussed in the literature is the following: direct contact of zinc oxide nanoparticles with the cell walls, leading to the destruction of membranes

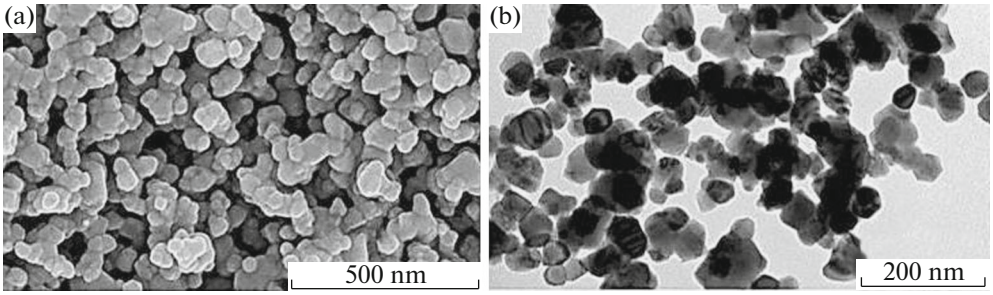

Fig. 1. Microphotographs of ZnO nanoparticles: (a) SEM and (b) TEM [3].

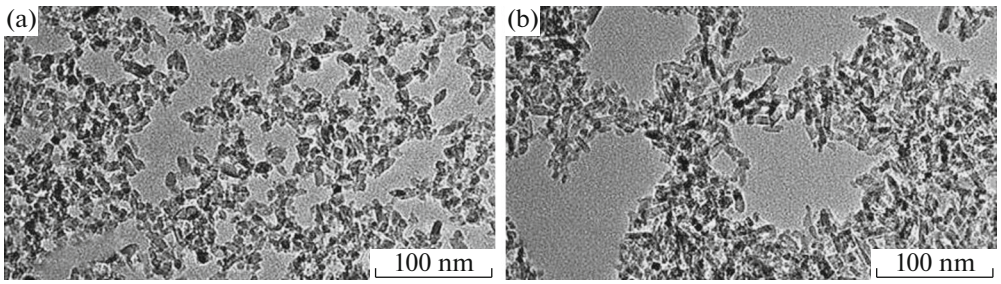

Fig. 2. TEM images of TiO_2 nanoparticles: (a) anatase and (b) rutile [4].

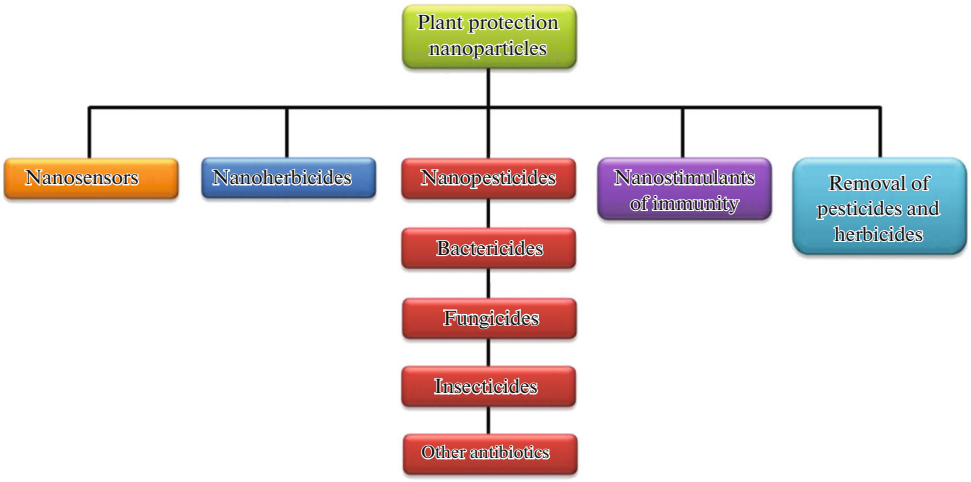

Fig. 3. (Color online) Directions for the use of nanoparticles in plant protection.

[6, 13–15], the release of antimicrobial ions (mainly Zn^{2+}), and the formation of reactive oxygen species [16, 17].

ZnO possesses the highest photocatalytic activity among all inorganic photocatalytic materials [18]. ZnO can effectively absorb UV radiation [19], and, therefore, its photoactivity increases; this feature significantly enhances the interaction between ZnO and bacteria. The activity of ZnO remains unchanged even after UV radiation is turned off, which is due to the electron depletion region owing to negative oxygen atoms (O^{-2} and O_2^{-2}) adsorbed on the surface [20]. Zinc oxide nanoparticles in an aqueous solution under UV radiation have a phototoxic effect based on the production of H^2O^2 and O^{2-} reactive oxygen species. A detailed reaction mechanism explaining this phenomenon was proposed earlier (Fig. 4) [11, 21, 22].

A higher antibacterial effect of zinc oxide nanoparticles was detected after UV irradiation against *Escherichia coli* and *Staphylococcus aureus* (98.65 and 99.45%, respectively) [23]. ZnO, however, possesses significant activity against bacteria under various test

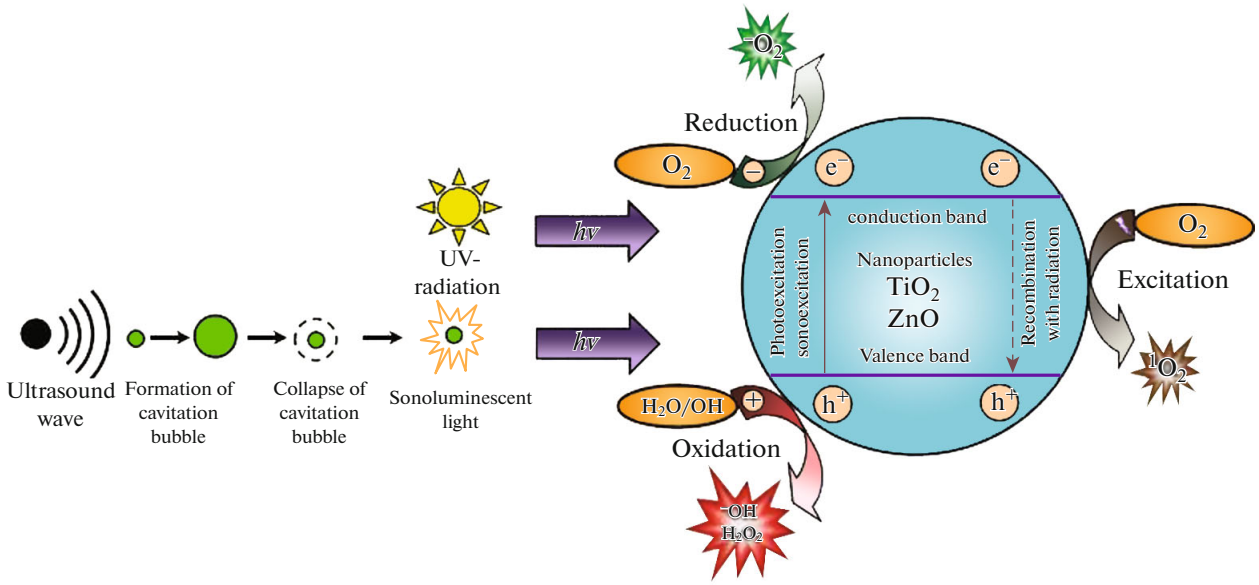

Fig. 4. (Color online) Mechanisms for the generation of reactive oxygen species [22].

conditions (conventional lighting and in the dark) [7, 24].

Many studies have shown that the various morphological parameters of ZnO nanoparticles, which are due to the synthesis conditions, influence the toxicity significantly [25, 27]. Desirable characteristics can be achieved by variation of the following parameters: the solvent, precursor, temperature, pH [28], and agents that regulate the form.

The antibacterial effect of ZnO nanoparticles in three different forms (nanorods, nanoflakes, and nanospheres) impregnated into low density polyethylene (LDPE) against *S. aureus* ATCC 25923 was studied [29]. An analysis performed according to ASTM E-2149 showed that ZnO nanospheres had the greatest inhibition of *S. aureus*. The shape of ZnO nanostructures can affect their internalization mechanism, because nanorods and nanowires can more easily penetrate bacterial cell walls than spherical nanoparticles [30]. At the same time, flower-shaped nanoparticles have a higher efficiency against *S. aureus* and *E. coli* than spherical and rod-shaped ones [31]. It was suggested that the polar faces of ZnO contribute to biochemical activity in addition to enhancement of internalization of zinc oxide nanoparticles through variation of the shape. In other words, more polar surfaces have a higher amount of oxygen vacancies. It is known that oxygen vacancies increase the generation of reactive oxygen species and, therefore, affect the photocatalytic activity of ZnO [31].

The antibacterial activity of nanoparticles correlates directly with their concentration and depends on the size of particles. A large surface area and a higher concentration enhance the antibacterial effect of ZnO nanoparticles [14, 32]. Smaller particles can easily penetrate bacterial membranes. The influence of the size (100–800 nm) of ZnO particles on their properties against *S. aureus* and *E. coli* was studied [33]. The authors found that antibacterial activity increases with a decrease in particle size. Similar effects were observed in other studies [7, 11, 14].

The size-dependent bactericidal activity was assessed [12]. The authors analyzed the reaction of a number of gram-negative and gram-positive strains. They found that the antibacterial activity of ZnO nanoparticles is inversely proportional to the particle size. An analysis of the growth and viability curves of bacteria showed that the activity of nanoparticles depends on the size; i.e., smaller particles have a greater antimicrobial effect under visible light. These data indicate that ZnO nanoparticles with a very small size (~12 nm) inhibited about 95% growth compared to that of the control. In addition, the influence of particles of various sizes (307, 212, 142, 88, and 30 nm) on bacterial growth at a concentration of 6 mmol was studied. The amount of viable cells decreased significantly with a decrease in particle size, which is due to the higher reactivity of small nanoparticles [11].

The authors also found that the antibacterial activity depends on the concentration and crystal structure of ZnO [34]. When the concentration was increased, bacterial survival decreased. A possible mechanism of toxicity, as the authors assumed, is violation of mitochondrial function, leakage of lactate dehydrogenase, and a change in the cell morphology under the action of nanoparticles.

The influence of the dispersion medium and the storage time of suspensions of zinc oxide nanoparticles

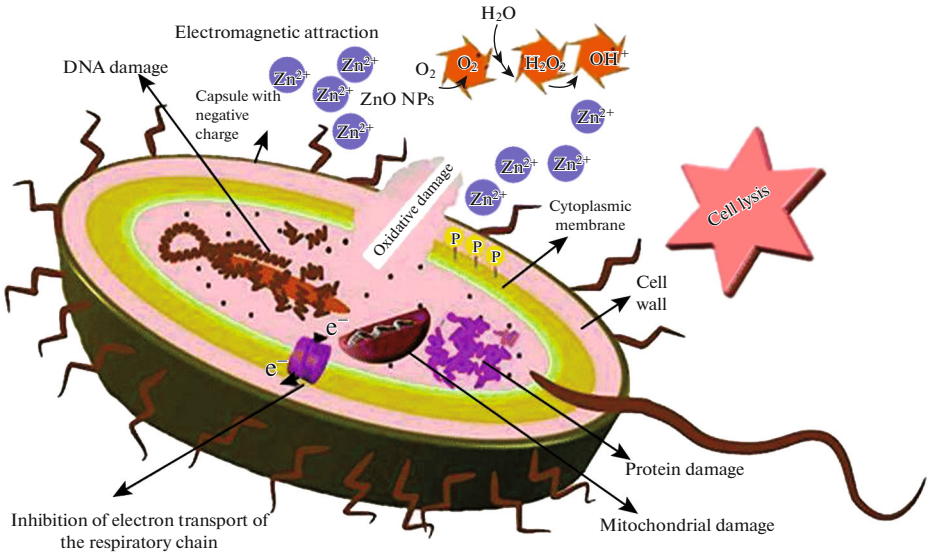

Fig. 5. (Color online) The mechanism of antibacterial action of ZnO nanoparticles on *S. typhi* as an example [36].

on their antibacterial activity against the *E. coli* luminescent strain was studied [35]. The authors found that freshly prepared aqueous dispersions of nanoparticles at concentrations of 1, 10, 100, and 1000 mg/L had the maximum activity: the survival rate was less than 5%; when the concentration decreased to 0.001 mg/l, the survival rate increased to 25%. After one day, the survival rate remained unchanged only at high concentrations (1, 10, 100, and 1000 mg/L), whereas it was 80–90% at lower concentrations. When the aqueous environment was replaced with physiological saline (0.9% NaCl), the survival rate was less than 5% only at 10, 100, and 1000 mg/L, regardless of the storage time; when the concentration of nanoparticles was decreased, the biocidal effect disappeared at 0.001 mg/L.

There is an electromagnetic attraction between negatively charged bacteria and positively charged ZnO nanoparticles to form bonds between them. ZnO nanoparticles interact with membrane lipids and thiol groups (–SH) of enzymes and proteins, which are important for bacterial respiration, transmembrane transport, and intracellular transport. In addition, ZnO nanoparticles can penetrate into bacterial cells and inactivate phosphorus and sulfur compounds, such as DNA and enzymes. The generation of reactive oxygen species (ROSs) plays a key role in this process, because damage to membranes, DNA, and cellular proteins is due to ROSs [36]. This process is shown schematically in Fig. 5.

Nanosized TiO_2 is also effective in suppressing bacteria [21, 37–41].

Its antibacterial activity depends on the light intensity [42], particle concentration and diameter [43, 44], ambient temperature [45], substrate chemical composition [46, 47], and species sensitivity of microorganisms [48, 49].

The influence of two TiO_2 anatase types (25 and 100 nm) on the bacterial community in a filter with biologically activated carbon was assessed with DNA analysis [50]. Both nanoparticle types significantly inhibited the level of bacterial adenosine triphosphate (ATP) ($p < 0.01$) and decreased the amount of copies of the 16S rDNA bacterial gene at 0.1 and 100 mg/L. At the same time, the diversity and uniformity of bacterial communities were significantly decreased. The relative amount of *Nitrospira* and *Betaproteobacteria* bacteria decreased after treatment with TiO_2, whereas the amount of *Bacilli* and *Gammaproteobacteria* bacteria increased. The size of TiO_2 particles had a greater effect on the bacterial composition than their concentration.

Hollow calcined nanospheres of titanium dioxide ($CSTiO_2$), with a size of about 345 nm and a shell thickness of 17 nm and obtained by electrospinning and subsequent deposition onto the atomic layer, were studied [51]. The antibacterial activity of $CSTiO_2$ was assessed by the inhibition of growth of *S. aureus* (ATCC®6538TM control strain together with MRSA 97-7 and MRSA 622-4 resistant strains) and *E. coli* (ATCC®25922TM control strain and *E. coli* 33.1 resistant strain). Commercial titanium dioxide nanoparticles were used in the experiment for comparison. The studies showed that $CSTiO_2$ had greater antibacterial activity against *S. aureus* and *E. coli* compared to that of commercial nanoparticles. At the same time, only $CSTiO_2$ had low antibacterial activity against *E. coli* MRSA 33.1 in the study with resistant bacteria. The authors assume that such a low efficiency is probably due to the high resistance of bacteria to a wide range of

exposure agents. UV radiation was used to enhance the antibacterial effect. After exposure for 60 min, the inhibitory effect of $CSTiO_2$ at a concentration of 100 μg/mL against *S. aureus* MRSA 97-7 increased significantly; no similar effect was observed for TiO_2 nanoparticles.

Fungicidal Action of ZnO and TiO_2 Nanoparticles

Numerous studies indicate that zinc oxide nanoparticles possess a fungicidal effect. Indeed, ZnO nanoparticles obtained by the sol–gel method with a 0.15 and 0.1 M precursor (zinc acetate dihydrate) inhibited the mycelial growth of the fungus *Erythricium salmonicolor* [52]. The inhibitory effect on fungal growth was studied by measuring the growth area as a function of time. Morphological changes were observed with high resolution optical microscopy (HROM), whereas transmission electron microscopy (TEM) was used to monitor changes in the ultrastructure. The results showed that the sample with a concentration of 9 mmol/L obtained from 0.15 M and 12 mmol/L for the 0.1 M system significantly inhibited the growth of *E. salmonicolor*. HROM images showed that there was a deformation in the growth structure: a noticeable thinning of hyphal fibers and a tendency to thicken. TEM results showed a liquefaction of the cytoplasmic contents, a decrease in its electron density in the presence of vacuoles, and significant damage to the cell wall.

The authors assessed the effects of ZnO nanoparticles on the viability of the pathogenic yeast *Candida albicans* [53]. They found that the effect of ZnO on the viability of *C. albicans* depends on the concentration. They also found that the minimum fungicidal concentration of ZnO is 0.1 mg/mL; it inhibited more than 95% of *C. albicans* growth. ZnO nanoparticles also inhibited the growth of *C. albicans* when added to the logarithmic phase of growth. The addition of histidine (an inactivator of hydroxyl radicals and singlet oxygen) decreased the effect of ZnO on *C. albicans* depending on the concentration. The antimycotic effect was almost completely eliminated after adding 5 mmol of histidine. The excitation of ZnO with visible light increased the death of yeast cells. These effects of histidine imply that ROSs play a significant role, including hydroxyl radicals and singlet oxygen, in cell death.

The antifungal activity of zinc oxide nanoparticles with a size of 70 ± 15 nm at concentrations of 0, 3, 6, and 12 mmol/L and the mechanism of their action against two pathogenic fungi (*Botrytis cinerea* and *Penicillium expansum*) were studied [54]. The results showed that ZnO nanoparticles at concentrations of more than 3 mmol/L can significantly inhibit the growth of *B. cinerea* and *P. expansum*. *P. expansum* was more sensitive to ZnO treatment than *B. cinerea*. Scanning electron microscopy (SEM) and Raman spectroscopy data showed that there are two different antifungal mechanisms of ZnO against *B. cinerea* and *P. expansum* (Figs. 6, 7). ZnO nanoparticles inhibited the growth of *B. cinerea*, affecting cellular functions, which led to the deformation of fungal hyphae. In the case of *P. expansum*, ZnO nanoparticles prevented the growth of conidiophores and conidia, which ultimately led to the death of hyphae [54].

The antifungal activity of ZnO nanoparticles obtained under various synthesis conditions was assessed against *Colletotrichum gloeosporioides* strains [55]. In vitro activity was found by calculation of the minimum inhibitory concentrations (MICs). A clear fungicidal effect was observed against two *C. gloeosporioides* strains that had led to anthracnose in avocados and papayas. The MICs to suppress the pathogen isolated from papaya were 0.156 and 0.312 mg/mL for the avocado fungus, regardless of the method to prepare the nanomaterial. The inhibition of radial growth of the mycelium in the presence of nanoparticles was 60, 70, and 80% at concentrations of 0.156, 0.312, and 0.624 mg/mL, respectively.

ZnO nanoparticles inhibit the growth of *Penicillium expansum* at 0.5 mmol; the fungicidal effect intensified with an increase in concentration, and the pathogen was almost completely suppressed at 15 mmol [56].

The first studies to assess the effectiveness of TiO_2 against fungi appeared in 1985 [57]. They proved that the amount of *Saccharomyces cerevisiae* cells inactivated in vitro after 240 min of UV-A irradiation increased from 72 to 98% in the presence of a 0.5% colloidal solution of TiO_2 nanoparticles. In addition, TiO_2 nanoparticles did not penetrate *S. cerevisiae* cells even after prolonged exposure, despite an increase in the ROS concentration in the cytosol [58]. These results indicate that the cell wall and plasma membrane were damaged insignificantly. The fungicidal properties of nanosized TiO_2 were also observed against other *C. albicans* fungi [21, 40, 59–62]. Moreover, recent studies showed that TiO_2 nanoparticles can be used to inactivate the mold species *Fusarium* sp. [41, 63], *Aspergillus niger* [21, 60, 64], and *Penicillium expansum* [65]. The relative resistance of fungi to photocatalytic oxidation is probably due to the protective effect of polysaccharides in the cell wall [66].

The effect of titanium dioxide nanoparticles on *Hypocrea lixii* (white rot) and *Mucor circinelloides* (brown rot), which are responsible for the rapid decay of wood, was studied [67]. The results showed that the photocatalytic activity of titanium dioxide nanoparticles prevents the fungal colonization of wood treated with suspensions of nanoparticles for a long time compared to untreated wood (Fig. 8).

Influence of ZnO and TiO_2 Nanoparticles on Plants

When nanoparticles are used as agents to sterilize explants, an important point is the analysis of their

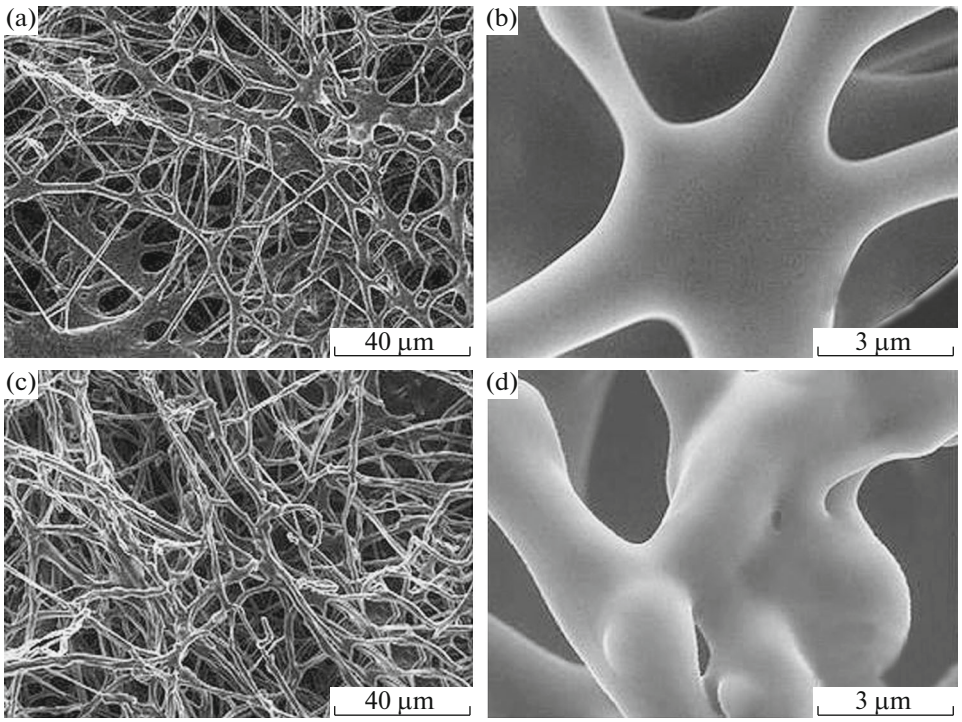

Fig. 6. SEM images of *Botrytis cinerea*: (a, b) control and (c, d) after treatment with ZnO [54].

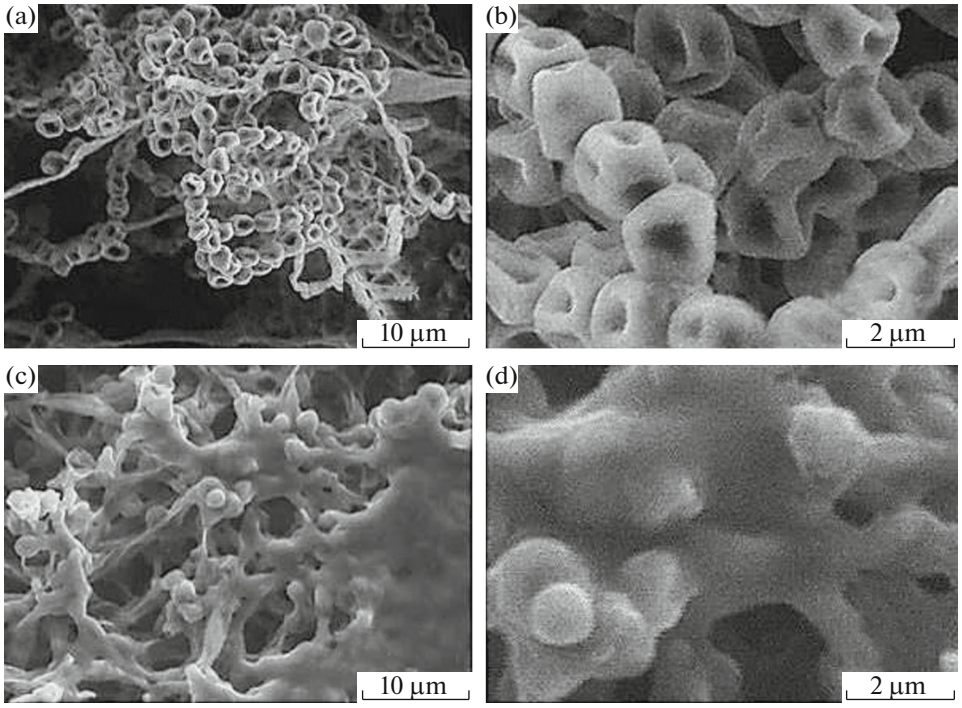

Fig. 7. SEM images of *Penicillium expansum*: (a, b) control and (c, d) after treatment with ZnO [54].

effect on plants. Zinc is an important essential element involved in many physiological processes in plants [68]. It is an integral component of special proteins (zinc fingers), which bind to DNA and RNA and contribute to their regulation and stabilization [69]. Zinc is an integral part of various enzymes, for example, oxidoreductases, transferase, and hydrolases [70], as well as ribosomes [71]. It plays an important role in the

formation of carbohydrates and chlorophyll and for the growth of plant roots [72].

At the same time, zinc oxide nanoparticles can negatively effect plant organisms. Indeed, the germination of corn seeds decreased under the influence of 2000 mg/L of ZnO nanoparticles [73]. The length of the roots and stems of wheat decreased by 35 and 30% under the action of zinc oxide nanoparticles at a concentration of 1000 mg/L, whereas the same parameters for cucumber plants decreased by 65 and 25%, respectively [74]. The biomass of buckwheat plants (*Fagopyrum esculentum*) decreased, and root cells were damaged under the action of 10–2000 mg/L of zinc oxide in the substrate [75].

Treatment with ZnO dispersion nanoparticles significantly inhibited the growth of tomato roots and shoots: the biomass decreased by about 10% after treatment of 400 mg/dm^3 of a substrate with ZnO and by 50% of plants treated with 800 mg/dm^3. The amount of chlorophylls *a* and *b* and the efficiency of photosynthesis also decreased. The authors assumed that toxicity was probably because of damage to the photochemical system, which limited photosynthesis and decreased biomass accumulation. ZnO nanoparticles also enhanced the transcription of genes of the antioxidant system, which is probably due to the fact that ZnO can enhance the protective response by an increase in the activity of antioxidant enzymes [76].

ZnO nanoparticles can affect the germination capacity of eggplant seeds depending on the cultivation medium [77]. Indeed, when seeds were germinated in the Murashige–Skoog medium, germination was inhibited with an increase in the concentration of nanoparticles from 5 to 20 mg/L and it decreased by more than 50% relative to the control at the maximum concentration. At the same time, germination in a peat medium was 100% at a concentration of nanoparticles of 20 and 100 mg/kg; when the concentration decreased to 5 mg/kg, germination decreased by 20%. Similar effects were observed for biomass growth. It especially should be noted that the maximum increase in length (~+25%) and mass (~+50%) of the root was observed when the concentration of peat was 100 mg/kg.

After treatment with 1000 and 1200 ppm of zinc oxide, 100% germination of seeds of corn plants was observed, whereas only 60% of the seeds germinated in the control. An increase in the concentration of nanoparticles to 1600 ppm, however, led to a decrease in the parameter to 40%. The authors also found that when the concentration of ZnO was 1200 ppm, there was a maximum increase in the plant biomass [78]. These results may be used to create conditions for better rooting of plants during clonal micropropagation and to transfer microclones from a test tube to soil conditions.

Onion plants treated with ZnO nanoparticles at a concentration of 20 and 30 μg/mL showed better growth and bloomed 12–14 days earlier than control

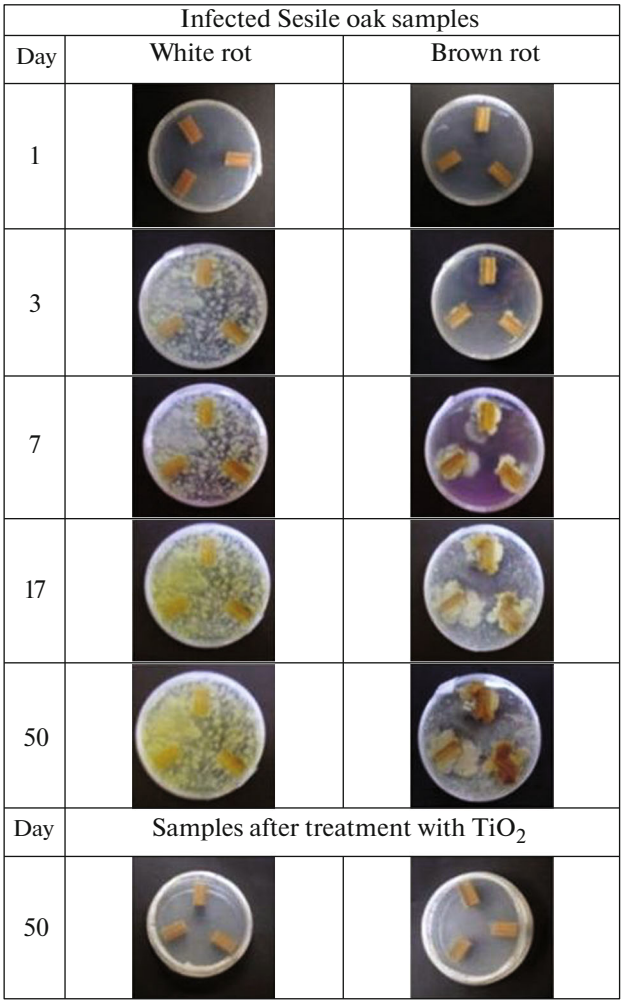

Fig. 8. (Color online) Mushroom growth on untreated and treated TiO_2 samples of Sessile oak [67].

plants [79]. The plants treated had higher values for seeds per umbel, seed weights per umbel, and 1000 seeds. Similar results indicate that ZnO nanoparticles can accelerate plant vegetation and provide better planting material.

The influence of ZnO nanoparticles on the biochemical parameters of safflower plants was studied [80]. The results showed that the amount of malondialdehyde increased at all concentrations of zinc oxide (10, 100, 500, and 1000 mg/L), which is probably due to the activation of free radical reactions in the cells. The amount of guaiacol peroxidase, polyphenol oxidase, and dehydrogenase increased at concentrations of 100, 10, 500, and 1000 mg/L, respectively; in addition, the amount of dehydrogenase decreased at other concentrations. These data indicate that antioxidant systems are activated in the presence of nanoparticles, which is probably due to stress for plants.

The growth characteristics, the activity of photosynthesis, and biomass of wheat plants increased pro-

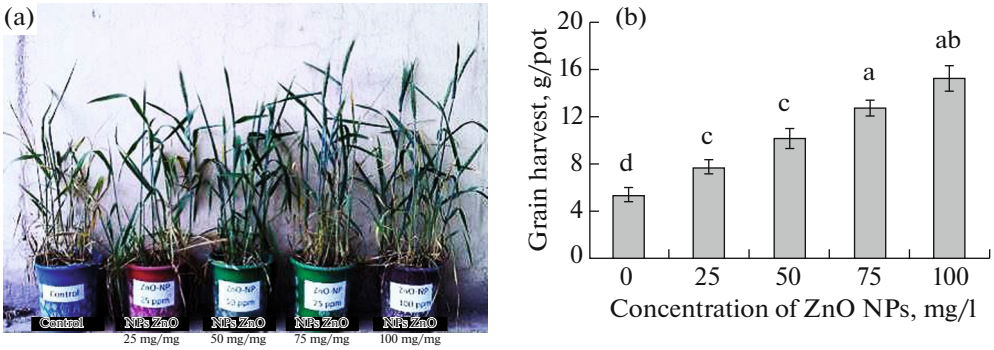

Fig. 9. (Color online) The influence of ZnO nanoparticles on wheat at concentrations of 25, 50, 75, and 100 mg/L [81].

portionally to the number of nanoparticles after treatment with ZnO nanoparticles at concentrations of 25, 50, 75, and 100 mg/L (Fig. 9) [81]. An analysis of zinc accumulation showed that its concentration also increased linearly compared to the control: by 25, 43, 51, and 65% in the shoots; by 20, 21, 29, and 43% in roots; and by 8, 35, 50, and 64% in grains [81].

Treatment with zinc oxide nanoparticles increased the rate of germination of capsicum seeds (*Capsicum annuum* L.) during the first seven days [82]. Germination increased by 12.50, 129.40, and 94.17% after treatment with ZnO suspensions of 100, 200, and 500 ppm, respectively. Analysis of the morphological parameters showed that treatment with nanoparticles did not have a significant effect on the development of the plume, but affected significantly ($p \leq 0.01$) the root length. The suspensions of nanoparticles (100, 200, and 500 ppm) inhibited the growth of roots and contributed to the accumulation of phenolic compounds in these organs.

The authors assessed the influence of various zinc compounds on the physiological reactions of habanero pepper plants (*Capsicum chinense* Jacq.) under greenhouse conditions [83]. They found that ZnO nanoparticles at a concentration of 1000 mg/L had a positive effect on plant height, stem diameter, and the amount of chlorophyll; it also increased the yield and biomass accumulation compared to that sample treated with $ZnSO_4$. Zinc oxide at a concentration of 2000 mg/L negatively affected the growth of plants, but significantly improved the quality of the fruit: the amount of capsaicin and dihydrocapsaicin increased by 19.3 and 10.9%, respectively; the Scoville Heat Units (SHUs) increased by 16.4%. In addition, ZnO nanoparticles at 2000 mg/L also increased the amount of total phenols and total flavonoids (soluble + bound) in fruits (14.50 and 26.9%, respectively).

The influence of titanium dioxide in macroforms and nanoforms on seed germination, morphometric parameters of seedlings, and photosynthetic pigments of peppermint (*Mentha piperita*) was studied [84]. The authors showed that titanium dioxide samples at concentrations of 100, 200, and 300 mg/L inhibited seed germination. The development of seedlings was also suppressed; an exception was the case when the concentration of TiO_2 nanoparticles was 100 mg/L, which led to an increase in the root length relative to the control. The amount of chlorophyll *a* and *b* increased under the action of TiO_2 nanoparticles by more than two times, regardless of the concentration. Macroform titanium dioxide had a positive effect only at 200 mg/L. The amount of carotenoids increased more than two times relative to the control after treatment with 100 mg/L TiO_2, whereas macroform titanium dioxide at 200 mg/L increased this parameter by more than three times.

The influence of TiO_2 nanoparticles on the production and quality of rosemary essential oil (*Rosmarinus officinalis*) was assessed [85]. The experimental treatment included the sputtering of TiO_2 nanoparticles in concentrations of 20, 40, 60, 100, 200, and 400 ppm on rosemary leaves. The results showed that the amount of many compounds in the essential oil with TiO_2 nanoparticles increased. This indicator, however, decreased at high concentrations (more than 200 ppm). An analysis of the amount of α-pinene, caryophyllene, and other compounds in the essential oil showed that it increased as much as possible, when the concentration of TiO_2 nanoparticles was 200 ppm.

The treatment of tomato seeds with suspensions of titanium dioxide nanoparticles (25 nm) at a concentration of 1000 mg/L significantly decreased the germination energy [86]. At the same time, there was no effect after the treatment of tomato seeds with TiO_2 dispersive nanoparticles (27 nm) at concentrations up to 4000 mg/L [87].

TiO_2 nanoparticles also inhibited the rate of seed germination of maize and Narbonne peas [88]. The seed germination of soft wheat plants decreased in the presence of anatase titanium dioxide at a concentration of 150 mg/L, whereas no such effect was observed, when anatase and rutile were mixed [89].

The authors found that TiO_2 anatase nanoparticles of about 3 nm in size penetrated into *Arabidopsis thali-*

Table 1. Biological action of ZnO and TiO_2 nanoparticles

Nanomaterial	Concentration	Size, nm	Effect	Test object	Ref.
Hydroxyapatite composite/ZnO			Inhibition: 98.65% 99.45%	 *E. coli* *S. aureus*	[23]
ZnO		100–800	Increase in antibacterial effect with decrease in particle size	*E. coli* *S. aureus*	[33]
	6 mmol	12, 30, 88, 142, 212, 307	Increase in antibacterial effect with a decrease in particle size. The inhibition is 95% for a particle size of 12 nm.	*S. aureus*	[12]
	0.1, 1, 10, 100, and 1000 mg/L	20–100 50–300	Survival is less than 5% at 1–1000 mg/L	*E. coli*	[33]
TiO_2	0.1 and 100 mg/L	25, 100	Inhibition of bacterial ATP level and decrease in the number of copies of the bacterial 16S rDNA gene	*Nitrospira* and *Betaproteobacteria* bacteria species	[50]
Titanium dioxide nanospheres ($CSTiO_2$)	100 μg/mL	345	Antibacterial effect	*S. aureus* *E. coli*	[51]
ZnO	9 and 12 mmol/L		Inhibition of growth	*E. salmonicolor*	[52]
	0.1 mg/mL	7–11	Inhibition of growth higher than 95%	*C. albicans*	[53]
	6 and 12 mmol/L	70 ± 15	Significant inhibition of growth	*B. cinerea* *P. expansum*	[54]
	0.5–15 mmol	<50	Increase in fungicidal effect with an increase in concentration. The suppression is almost complete at 15 mmol.	*P. expansum*	[56]
TiO_2	0.1 g/L	30	Degradation of the cell wall, destruction of cell compartments, and increase in the concentration of ROS in cytosol	*S. cerevisiae*	[58]
	35 mg/L	20	Inhibition of growth under UV irradiation	*F. verticillioides* *F. oxysporum* *F. solani* *F. anthophilum* *F. equiseti*	[63]
	0.25 mg/mL		Growth inhibition of colony	*Hypocrea lixii* *Mucor circinelloides*	[67]

Table 1. (Contd.)

Nanomaterial	Concentration	Size, nm	Effect	Test object	Ref.
ZnO	2000 mg/L		Decrease in germination rate of seeds	*Zea mays*	[73]
	1000 mg/L		Suppression of growth of roots and stems	*Triticum*	[74]
	10–2000 mg/L	<50	Decrease in biomass and root cell damage	*Fagopyrum esculentum*	[75]
	400 and 800 mg/dm^3		Decrease in biomass and suppression of root and shoot growth	*Solanum lycopersicum*	[76]
	5–20 mg/L 5–100 mg/kg		Inhibition of germination with an increase in the concentration of nanoparticles in MS and an increase in germination with an increase in the concentration in peat. Stimulation of growth of roots and stems in a peat environment at maximum concentration	*S. melongena*	[77]
	1000 and 1200 ppm		Increase in germination and biomass	*Zea mays*	[78]
	20 and 30 μg/mL		Acceleration of development and stimulation of productivity	*Allium cepa*	[79]
	25, 50, 75, and 100 mg/L	34.4	Increase in growth characteristics, photosynthesis, and plant biomass activity is proportional to an increase in concentration	*Triticumaestivum*	[81]
	100, 200, and 500 ppm	12–24	Increase in germination	*Capsicum chinense Jacq*	[82]
TiO_2	100, 200, and 300 mg/L		Decrease in germination and an increase in root length with minimal concentration. Increase in chlorophyll *a* and *b*	*Mentha piperita*	[84]
	20, 40, 60, and 100 ppm		Increase in thte amount of essential oil	*Rosmarinus officinalis*	[85]
	10 mg/L	21	Germination acceleration and growth stimulation	*T. aestivum* L. var. Pishtaz	[92]

ana cells and accumulated in vacuoles and nuclei of root cells and vacuoles or structures similar to endosomes in hypocotyl, cotyledon, and leaf cells [90]. Although this internalization of TiO_2 nanoparticles did not affect the cell viability and morphology, the authors assumed that the absorption and distribution of nanoparticles lead to cellular and molecular changes. Further studies showed that TiO_2 ultrafine anatase nanoparticles led to reorganization and elimination of microtubules of *Arabidopsis thaliana* with subsequent high degradation of tubulin monomers depending on proteasome. TiO_2 nanoparticles induce the isotropic growth of root cells like any other microtubule-destroying agents [91].

Moreover, some authors showed that titanium dioxide nanoparticles had positive effect on plant growth. Indeed, TiO_2 at a concentration of 10 mg/L accelerated the germination of wheat seeds by 34% and contributed to a significant improvement in plant growth [92].

CONCLUSIONS

This review showed that ZnO and TiO_2 nanoparticles can be used successfully as antimicrobial agents, and their biological effect depends on certain factors: photocatalytic activity, particle size, concentration, morphology, and surface modification (Table 1). The toxicity mechanisms, the primary one of which is the generation of reactive oxygen species leading to oxidative stress, are also due to these factors.

The data concerning the direct effect of ZnO and TiO_2 nanoparticles on plants, however, are contradictory, which is probably due to the various particle shapes and sizes, their concentrations, and species characteristics of plants. Thus, the studies confirm that photocatalytically active ZnO and TiO_2 nanoparticles may be used effectively as bactericidal and fungicidal drugs for sterilizing explants during clonal micropropagation of plants, but taking into account the possible phytotoxicity of these particles, which requires further study.

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