Scientific Papers. Series A. Agronomy, Vol. LXIV, No. 2, 2021
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

ASSESSMENT OF ANTIFUNGAL ACTIVITY OF GOLD-CHITOSAN AND CARBON NANOPARTICLES AGAINST *Rhizoctonia solani* Kühn

Florin Daniel LIPŞA[1], Elena Laura URSU[2], Andrei Mihai GAFENCU[1], Mihaela Andreea FLOREA[1], Ana CAZACU[1]

[1]"Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine Iasi, 3 Mihail Sadoveanu Alley, Iasi, Romania
[2]"Petru Poni" Institute of Macromolecular Chemistry, 41A Grigore GhicaVoda Alley, Iasi, Romania

Corresponding author email: anacazacu@uaiasi.ro

Abstract

Nanoparticles are implemented in different biotechnological fields, and there is interest in their use in plant biology. Nanotechnology can help overcome the persistent limitations of using conventional fungicides in the management of plant diseases, contributing to a safer environment. Hence, this study is focused on evaluating the behavior of nanoparticles on Rhizoctonia solani, which has a worldwide distribution and causing important economic losses to many agricultural and horticultural crop species. Rhizoctonia solani (teleomorph: Thanatephorus cucumeris) is an basidiomycetous fungus that is well-known as a soil borne plant pathogen, adapted to any soil type, and it lives in different forms on plant debris. Gold-chitosan and carbon nanoparticles were suspended in malt extract peptone agar nutritive media, and their antifungal activity was evaluated at 24, 48, 72 and 96 hours after incubation by measuring the diameter of fungal colonies. The results showed that gold-chitosan nanoparticles have antifungal properties against R. solani, the fungal colony growth diameter being reduced. Likewise, it was observed that the colony diameter was smaller when the nanoparticle concentration increased. However, the highest carbon nanoparticle concentration applied during the experiment's execution was not able to inhibit R. solani growth.

Key words*: gold-chitosan nanoparticles, carbon nanoparticles, antifungal activity, Rhizoctonia solani.*

INTRODUCTION

Temperature and humidity play important roles in plant biology during the growth of plants in the field. Filamentous fungi can harm and degrade plants at any time during their phenophases or after harvesting, reducing the germination potential of seeds or the nutritional value of plant products (Sturrock et al., 2015).

Rhizoctonia solani (teleomorph: *Thanatephorus cucumeris*) is one of the most destructive pathogens infecting a wide range of crops around the world and causes a wide range of plant diseases (e.g. black scurf of potato, bare patch of cereals, tomato foot and root rot, root rot of sugar beet, belly rot of cucumber, damping off in soybean seedling) (Akgun et al., 2018; Nikraftar et al., 2013). The pathogen is not efficiently controlled by resistance breeding and is necessary to plan other effective disease control strategies in crop protection beside conventional fungicides. One of the strategies is the usage of new antifungal substances in order to control and inhibit fungal growt.

Antifungal materials, which may also serve as biostimulants, allowing plants to expand (through different processes) when used in limited amounts, are an interesting group of materials. Nanoparticles (NPs) are a type of biostimulant that have a high density of surface charges that interact with the surface charges found on cell walls and membranes (Juárez-Maldonadoet al., 2019). Furthermore, because of the various possibilities for modifying the combination of their physical and chemical properties, NPs have begun to be used as new forms of antimicrobial agents. The antimicrobial activity of NPs is related to their association with functional groups on the surface of microorganism cells, which results in the inactivation of the microorganism (Cui et al., 2012). Just a few reports on the effects of gold or carbon nanoparticles on bacteria (Perni et al., 2009) or fungi (Ahmad et al., 2013; Lipsa et al.,

2020) have been published. Chemical reduction, sol-gel processes, gas condensation, electrodeposition or vacuum deposition and vaporization, and pulsed laser ablation in liquid (PLAL) can all be used to create NPs. To extract NPs from a bulk solution, a reducing agent (e.g., sodium borohydride, ascorbic acid, sodium citrate, amino acids) is used. PLAL is a flexible technique for making surfactant-free stable colloidal solutions of nanoparticles from a variety of materials (Zeng et al., 2012). Gold nanoparticles were synthesized using chitosan (AuNPs–chitosan), which acts as both a reducing and stabilizing agent, and carbon nanoparticles (CNPs) were synthesized using PLAL. Due to their inert nature, gold NPs are considered to be nontoxic, unlike other inorganic nanoparticles (Lipsa et al., 2020; Rahimi et al., 2019).

Carbon nanomaterial quality assurance research is gaining traction (Ursu et al., 2019; 2020), with the aim of facilitating their use in a variety of applications (Chung et al., 2011), including plant science, drug delivery systems, energy storage devices, bioimaging, and biosensors. Carbon nanomaterials come in a variety of forms (e.g., carbon nanotubes, carbon nanoparticles, and carbon dots), and although they provide a number of benefits, their possible toxicity to the environment is a significant consideration (Poland et al., 2008). However, depending on the experimental procedure used to create them, certain carbon nanomaterials can be toxic while others are not (Firme et al., 2010). As a result, researchers are looking for new ways to make nontoxic carbon nanoparticles with the aim of using them safely to boost crop production (Zaytseva & Neumann, 2016).

The purpose of this study is to evaluate the fungicidal activit of AuNPs-chitosan and CNPs on *Rhizoctonia solani*.

MATERIALS AND METHODS

Synthesis of Chitosan-Stabilized Gold Nanoparticles

Solutions of tetrachloroauric acid (HAuCl4; 0.01 M) and 0.1 mg/mL chitosan were combined in different ratios and then treated with an ultrasonic field (for 20 minutes at a temperature of 55°C to produce AuNPs-chitosan with concentrations of 25, 50, and 75 g/mL. The gold concentration was used to mark the samples. AuNP25, for example, is the name given to a sample of AuNPs-chitosan with a concentration of 25 g/mL.

Synthesis of Carbon Nanoparticles

The PLAL method was used to create stable CNP suspensions in ethanol that were free of surfactants. As a result, two CNP solutions for two laser fluences of 2 and 3 J/cm were obtained, resulting in samples labeled CNP 1 and CNP 2, respectively. CNP 1 had a concentration of 19 mg/mL, while CNP 2 had a concentration of 23 mg/mL.

Nanoparticles Application to Fungi

The Leibniz Institute German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) provided the *Rhizoctonia solani* DSM 22844 strain used in this research. The phytopathogenic fungi were routinely grown at 28°C in the climatic chamber on malt extract-peptone-agar (MEPA) medium containing 30 g malt extract, 3 g soya peptone and 15 g agar (Merck, Germany).

The antimicrobial properties of AuNPs-chitosan and CNPs against *R. solani* strain were studied at different concentrations, ranging from 25 to 75 g/mL for AuNPs-chitosan and 19 to 23 mg/mL for CNPs.

The gold and carbon-based NPs were suspended in 15 mL MEPA medium and poured into Petri dishes with a diameter of 90 mm. The NP solutions were applied to Petri dishes in various doses (0, 0.5, 1, 2, and 5 mL) in order to determine the most effective dose for each NP solution (regardless of the initial concentration of the respective solution). Using a media preparator with automatic dispenser (Masterclave 09 + APS 320/90 AES Laboratoire, France) for Petri plates, the MEPA medium was prepared and poured.

After cooling and solidification, the plates were inoculated aseptically with 7 mm diameter disks of *R. solani* taken from an actively developing edge of a five-day-old culture and incubated at 28°C for 4 days, supplemented with different concentrations of NPs.

Every 24 hours, the fungal plaque diameter from the inoculated plates was determined, and the mycelial development was photographed. For each microbial determination, the

procedure was carried out three times. To measure the percentage of growth inhibition, the obtained values were compared to those of the control (without NPs) using Formula (1):

$$\% \text{ Inhibition rate} = \frac{(Mc - Mt)}{Mc} \times 100, \quad (1)$$

where Mc is the mycelial growth for the control plate, and Mt is the mycelial growth for the plates treated with different dosage of NP solutions. Values are shown in terms of mean and standard deviation (mm).

Statistical Analysis

A two-sample t-test with unequal variances was used to assess the effect of nanoparticles on *R. solani* strain using Microsoft Excel 2016 software (Microsoft, USA). The differences were considered statistically important at the 0.05 likelihood level (p 0.05) when the values were compared to the average of all the values in the experiment.

RESULTS AND DISCUSSIONS

The aim of this study was to determine the inhibitory action of AuNPs-chitosan and CNPs on colony formation from the mycelia of *R. solani* under laboratory conditions (*in vitro*). Figure 1 shows the growth rate of *R. solani* strain in the presence of the measured NPs.

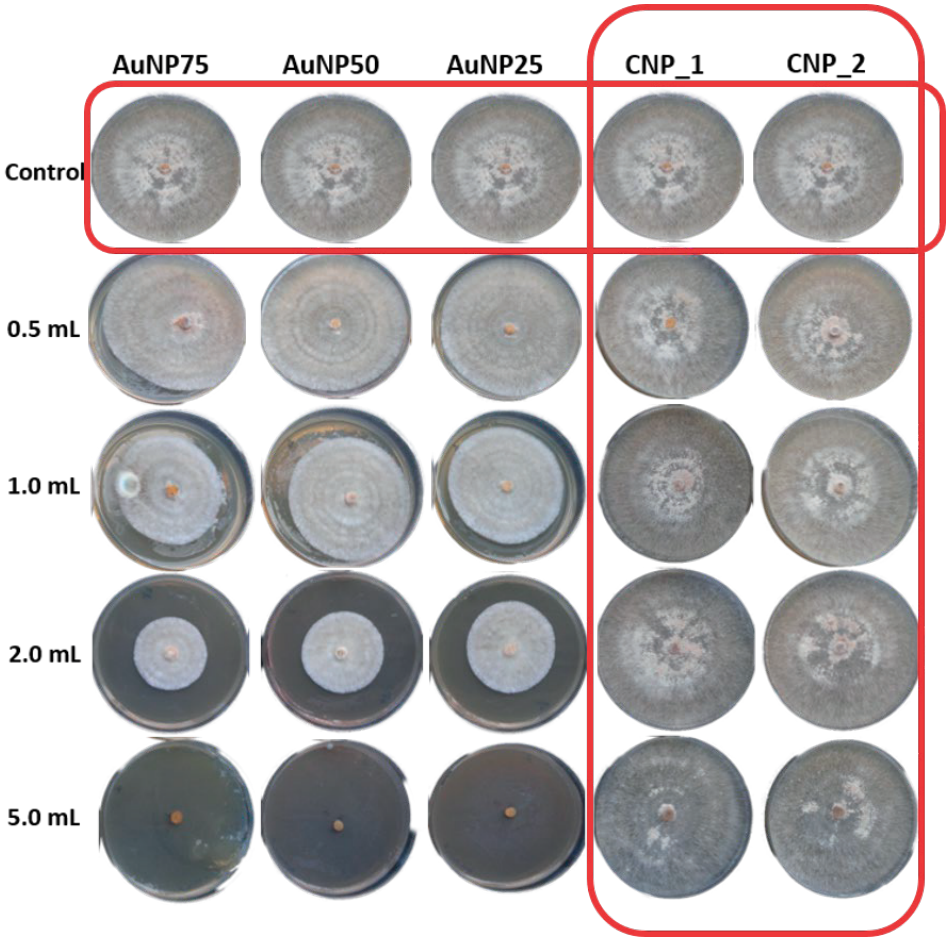

Figure 1. Effects of the interactions of different nanoparticles at different concentrations and doses on the inhibition of mycelial growth of *Rhizoctonia solani* DSM 22844 strain. There was no inhibition in the case of the control plates. For all the controls, the same picture was used as there was no difference between the control plates.

The tested NPs inhibited radial growth of *Rhizoctonia solani* strain in different ways at different concentration levels. When compared to the lowest dosage (0.5 mL), AuNPs-chitosan exhibited inhibitory effects at the maximum dosage (5 mL). Figures 1 and 2 show the impact of AuNPs-chitosan and CNP concentrations on the mycelial growth of *R. solani* strain tested after 4 days of incubation. In case of CNPs no inhibitory effects were observed and no information are present in Figure 2.

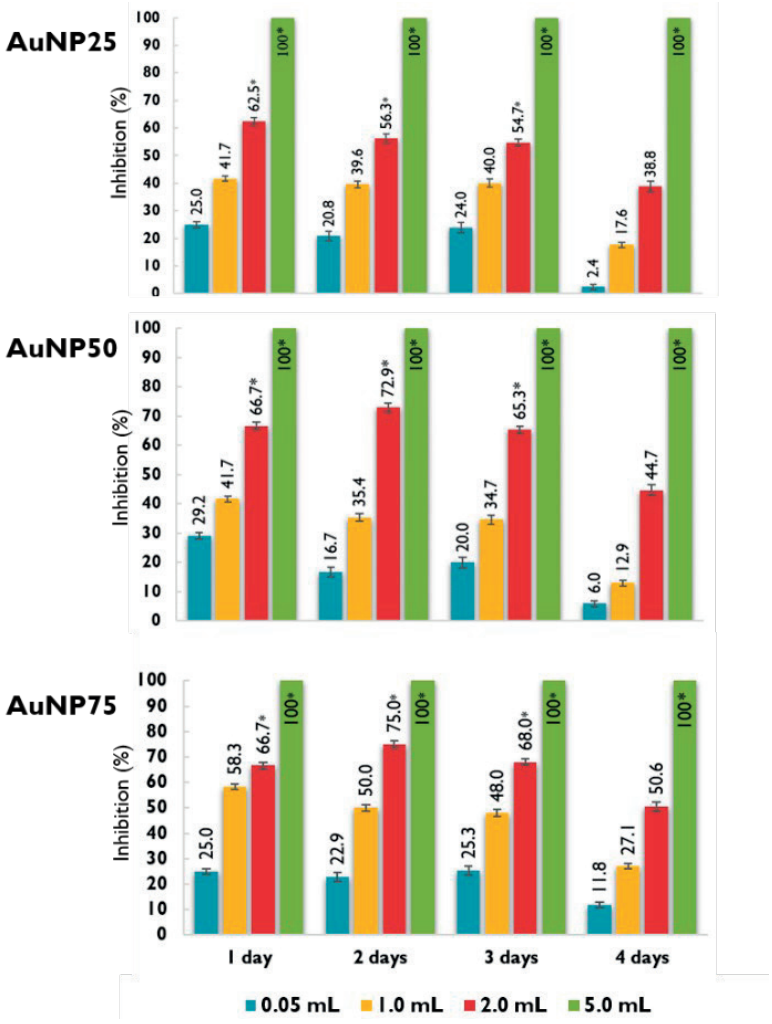

Figure 2. Antifungal activity of gold-chitosan (AuNPs-chitosan) nanoparticles applied at different concentrations and dosage on *Rhizoctonia solani* DSM 22844 strain on MEPA medium after 1, 2, 3, and 4 days of incubation at 28°C. *Rhizoctonia solani* DSM 22844 strain with different concentrations, ranging from 25 to 75 µg/mL in the case of AuNPs-chitosan. The percentages of growth inhibition were calculated in relation to the control. Error bars represent a standard deviation from the mean ($n = 3$). Significant differences ($p < 0.05$) are marked with an asterisk.

The application of AuNPs-chitosan at different concentrations and dosage was effective on *R. solani* strain; in the case of the pathogen, a mycelial growth inhibition rate of 100% ($p < 0.05$) was observed. Absolute inhibitions were obtained through the application of 5.0 mL AuNPs-chitosan for all three different concentrations (i.e. AuNP25, AuNP50, and AuNP75), the measurements being made after 1, 2, 3, and 4 days, respectively. Furthermore, a decrease in mycelial growth inhibition was observed throughout the four-day trial period

when AuNPs-chitosan concentrations and dosages were lower. In the case of the *R. solani* DSM 22844 strain, the results shown in Figure 2 revealed that AuNP25 had no antifungal activity at a dosage of 0.5 mL. In almost all cases, the efficacy of all gold–chitosan nanoparticles is observed to increase with dosage and concentration. The antifungal activity of AuNPs-chitosan against the plant pathogen could be due to a synergistic effect between gold nanoparticles and chitosan.

Antifungal activity of gold nanoparticles against plasma membrane proteins is size dependent (Ahmad et al., 2013). NPs can cause activity loss by interacting directly with enzymes involved in the regulation of the proton gradient across the plasma membrane. The fungal membrane will then be unable to regulate H+ transport, resulting in cell growth retardation and death (Beyenbach & Wieczorek., 2006). AuNPs can diffuse through the cell membrane and interact with sulfur-containing proteins in the membrane or phosphorus-containing bases in the cells' DNA to inhibit synthesis, reparation, and replication, leading to cell death, according to another study (Tan et al., 2011).

Chitosan is among the most promising plant pathogen-fighting compounds. It may increase antimicrobial activity by binding to the cell surface and inhibiting pathogen growth alone or in combination with other nanoparticles. Chitosan's antimicrobial activity is affected by its form, molecular weight, and concentration. Chitosan with a low molecular weight can pass through the fungal membrane and inhibit microbial growth. Chitosan attaches to the cell surface of microorganisms at low concentrations, causing membrane disruptions and ultimately the death of the microbial cell due to leakage of intracellular components (Hosseinnejad & Jafari, 2016).

Another part of our study consists of using CNPs at different concentrations (CNP_1 = 19 mg/mL, CNP_2 = 23 mg/mL) and doses (0.5, 1, 2, and 5 mL) to analyze the inhibitory effects on *R. solani* DSM 22844 strain. From Figure 1, it can be observed that 4 days after inoculation and at the highest dosage (5 mL per Petri dish), no inhibition in the case of the DSM 22844 strain was attained.

In the case of both CNPs (CNP_1 and CNP_2), despite the fact that the dimension of CNPs were different (CNP_2 = 97 nm vs. CNP_1 = 120 nm in diameter), the results showed no influence on the mycelia growth, regardless of the exposure doses, on *R. solani* DSM 22844 strain (data and figure not presented).

No inhibition on growth rates was registered because the effects of CNPs within different biological systems (plants, microorganisms) are diverse and dependent on the CNP type, its physical characteristics, type of organism, dosage/concentration, method of application, and duration of the exposure. In plants, Verma et al. (2019) found that CNPs were successful at enhancing water uptake and transport, seed germination, activating water channel proteins, and promoting nutrient absorption at lower concentrations. All of these improvements were absent when the CNP concentration was high.

In *R. solani* strain, statistically significant ($p < 0.05$) mycelial growth inhibition was observed using the findings presented above for AuNPs–chitosan. Significant differences were found for 2-mL and 5-mL NP dosage (Figure 2).

CONCLUSIONS

In agricultural fields, the use of AuNPs-chitosan is a promising alternative to the typical use of conventional fungicides to combat plant pathogens like *Rhizoctonia solani*. The current study shows that AuNPs-chitosan has antifungal activity against *R. solani*, one of the most destructive pathogens infecting a wide range of crops around the world.

The application of 5 mL of AuNPs-chitosan solution to the *Rhizoctonia solani* DSM 22844 strain resulted in absolute inhibition ($p < 0.05$) for all concentrations tested (25, 50, and 75 g/mL). Also, a dosage of 2 mL AuNPs-chitosan solution presented a statistically significant differences in the first 3 days of this study. Our findings show that the dosage used when evaluating the antifungal efficacy of an NP solution is an important factor to consider (regardless of the concentration used). As a result, instead of using a high-concentration NP solution (which means higher manufacturing costs), a lower-concentration solution can be used if the correct dose is known.

To summarize, particle size, molecular weight, concentration, and dosage are essential factors that should be considered in the future developing of new fungicide formulations with applications in plant disease management. Therefore, more investigations on field applications (*in vivo*) are needed.

ACKNOWLEDGEMENTS

This research was funded by the CNCS-UEFISCDI, PN-III-P1-1.1-TE-2016-2336 project.

REFERENCES

Ahmad, T., Wani, I.A., Lone, I.H., Ganguly, A., Manzoor, N., Ahmad, A., Ahmed, J., Al-Shihri, A.S. (2013). Antifungal activity of gold nanoparticles prepared by solvothermal method. *Materials Research Bulletin*, *48*, 12–20.

Akgun, I., Ayata, R., Karaman, R., Karaca, G. (2018). Effect of wheatgrass (*Triticumaestivum*L.) juice on seedling growth and *Rhizoctonia solani* on corn. *Scientific Papers. Series A. Agronomy*, *LXI*(1), 149–154.

Beyenbach, K.W., Wieczorek, H. (2006). The V-type H+ ATPase: Molecular structure and function, physiological roles and regulation. *Journal of Experimental Biology*, *209*. 577–589.

Chung, H., Son, Y., Yoon, T.K., Kim, S., Kim, W. (2011). The effect of multi-walled carbon nanotubes on soil microbial activity. *Ecotoxicology and Environmental Safety*, *74*, 569–575.

Cui, Y., Zhao, Y., Tian, Y., Zhang, W., Lü, X., Jiang, X. (2012). The molecular mechanism of action of bactericidal gold nanoparticles on *Escherichia. coli*. *Biomaterials, 33*, 2327–2333.

Firme, C.P., Bandaru, P.R. (2010). Toxicity issues in the application of carbon nanotubes to biological systems. *Nanomedicine: Nanotechnology, Biology, and Medicine*, *6*, 245–256.

Hosseinnejad, M., Jafari, S.M. (2016). Evaluation of different factors affecting antimicrobial properties of chitosan.*International Journal of Biological Macromolecules*, *85*, 467–475.

Juárez-Maldonado, A., Ortega-Ortíz, H., Morales-Díaz, A.B., González-Morales, S., Morelos-Moreno, Á., Cabrera-De la Fuente, M., Sandoval-Rangel, A., Cadenas-Pliego, G., Benavides-Mendoza, A. (2019). Nanoparticles and nanomaterials as plant biostimulants. *International Journal of Molecular Sciences*, *20*, 162.

Lipsa, F.D., Ursu, E.L., Ursu, C., Ulea, E., Cazacu, A. (2020). Evaluation of the Antifungal Activity of Gold–Chitosan and Carbon Nanoparticles on *Fusarium oxysporum*. *Agronomy*, *10*, 1143.

Nikraftar, F., Taheri, P., Rastegar, M.F., Tarighi, S. (2013). Tomato partial resistance to *Rhizoctonia solani* involves antioxidative defense mechanisms. *Physiological and Molecular Plant Pathology*, *81*, 74–83.

Perni, S., Piccirillo, C., Pratten, J., Prokopovich, P., Chrzanowski, W., Parkin, I.P., Wilson, M. (2009). The antimicrobial properties of light-activated polymers containing methylene blue and gold nanoparticles. *Biomaterials, 30*, 89–93.

Poland, C.A., Duffin, R., Kinloch, I., Maynard, A., Wallace, W.A.H., Seaton, A., Stone, V., Brown, S., MacNee, W., Donaldson, K. (2008). Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. *Nature Nanotechnology*, *3*, 423–428.

Rahimi, H., Roudbarmohammadi, S., Delavari, H.H., Roudbary, M. (2019). Antifungal effects of indolicidin-conjugated gold nanoparticles against fluconazole-resistant strains of *Candida albicans* isolated from patients with burn infection. *International Journal of Nanomedicine*, *14*, 5323–5338.

Sturrock, C.J., Woodhall, J., Brown, M., Walker, C., Mooney, S.J., Ray, R. V. (2015). Effects of damping-off caused by *Rhizoctonia solani* anastomosis group 2-1 on roots of wheat and oil seed rape quantified using X-ray Computed Tomography and real-time PCR. *Frontiers in plant science*, *6*, 461.

Tan, Y.N., Lee, K.H., Su, X. (2011). Study of single-stranded DNA binding protein–nucleic acids interactions using unmodified gold nanoparticles and its application for detection of single nucleotide polymorphisms. *Analytical Chemistry*, *83*, 4251–4257.

Ursu, C., Nica, P., Focsa, C. (2018). Excimer laser ablation of graphite: The enhancement of carbon dimer formation. *Applied Surface Science*, *456,* 717–725.

Ursu, C., Nica, P., Rusu, B.G., Focsa, C. (2020). V-shape plasma generated by excimer laser ablation of graphite in argon: Spectroscopic investigations. *SpectrochimicaActa Part B: Atomic Spectroscopy*, *163*, 105743.

Verma, S.K., Das, A.K., Gantait, S., Kumar, V., Gurel, E. (2019). Applications of carbon nanomaterials in the plant system: A perspective view on the pros and cons. *Science of the Total Environment*, *667*, 485–499.

Zaytseva, O., Neumann, G. (2016). Carbon nanomaterials: Production, impact on plant development, agricultural and environmental applications. *Chemical and Biological Technologies in Agriculture*, *3*, 17.

Zeng, H., Du, X.-W., Singh, S.C., Kulinich, S.A., Yang, S., He, J., Cai, W. (2012). Nanomaterials via laser ablation/irradiation in liquid: A review. *Advanced Functional Materials*, *22*, 1333–1353.