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EVALUATION OF STUDIES DETERMINING THE EFFECTS OF AgNPs ON WHEAT SEED GERMINATION ACCORDING TO US EPA AND OECD GUIDELINES: SYSTEMATIC REVIEW

AgNP'LERİN BUĞDAY TOHUMU ÇİMLENMESİNE ETKİLERİNİ BELİRLEYEN ÇALIŞMALARIN US EPA VE OECD YÖNERGELERİNE GÖRE DEĞERLENDİRİLMESİ: SİSTEMATİK DERLEME

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ABSTRACT

Silver nanoparticles (AgNPs) are used in many industries due to their unique properties, especially for microbial activity. For that, most of the scientific studies focus on the antimicrobial effects of AgNPs. However, there is a lack of information about the effects of AgNPs on the growth of plants, especially commonly cultivated wheat plants over the last decades. In this systematic review, we tried to examine the selected studies determining the effects of AgNPs on seed germination of wheat. This research was focused on scientific researches published from 2009 to 2019. The reviewing process has been conducted by 3 keywords and 4 combinations of them in 4 different databases according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). Among the 35453 screening records, 7 articles were obtained according to the selection criteria. Obtained results from these 7 articles showed that commercially obtained or chemically synthesized AgNPs have adverse effects on seed germination of wheat than green synthesized AgNPs.

Keywords: Silver nanoparticles, green synthesise, wheat, phytotoxicity

ÖZET

Gümüş nanoparçacıklar (AgNP'ler) benzersiz özellikleri nedeniyle, özellikle mikrobiyal aktivite nedeniyle birçok endüstride kullanılmaktadır. Bu yüzden bilimsel çalışmaların çoğu AgNP'lerin antimikrobiyal etkileri üzerine odaklanmıştır. Bununla birlikte, AgNP'lerin son on yılda bitkilerin, özellikle yaygın olarak yetiştirilen buğday bitkilerinin büyümesi üzerindeki etkileri hakkında bilgi eksikliği vardır. Bu sistematik derlemede, AgNP'lerin buğdayın tohum çimlenmesi üzerindeki etkilerini belirleyen seçilmiş çalışmaları incelemeye çalıştık. Bu çalışmada 2009-2019 yılları arasında yayınlanan bilimsel araştırmalara odaklanmıştır. İnceleme süreci, Sistematik İncelemeler ve Meta Analizler için Tercih Edilen Raporlama Öğeleri (PRISMA)ne göre 4 farklı veritabanında 3 anahtar kelime ve bunların 4 farklı kombinasyonu ile yürütülmüştür. 35453 tarama kaydı arasından seçim kriterlerine göre toplamda 7 makale elde edilmiştir. Seçilen 7 makale incelendiğinde, ticari olarak satın alınan veya kimyasal olarak sentezlenen AgNP'lerin buğdayın tohum çimlenmesi üzerinde yeşil sentezlenmiş AgNP'lere göre olumsuz etkilerinin olduğu belirlenmiştir.

Anahtar Kelimeler: Gümüş nanopartikülleri, yeşil sentez, buğday, fitotoksisite

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INTRODUCTION

Engineered nanoparticles are designed at the molecular or atomic level. For the benefit from the advantages being offered by their small size and thus unique properties, compared to their bulk forms are started to use widely. Since nanotechnology has developed rapidly, many nanoparticle types are used as raw materials or by-products in many industries. Therefore, there is a need to investigate the effects of these nanoparticles on living organisms.

Silver nanoparticles (AgNPs) are commonly used in many industries, due to their antimicrobial, medicinal, electrical and catalytic activities (Galazzia, Júnior, de Lima, Gozzo & Arruda, 2019; Rashid, Azeem, Khan, Shah & Ahmad, 2019). According to Lee, Kwak and An (2012) and Galazzia et al. (2019), the silver nanoparticles are the most used nanoparticles among the other metallic nanoparticles, as 10 times more. Thus, AgNPs have gained popularity for researchers and producers, especially in the agricultural sector and also in food products. Unfortunately, the usage of AgNPs in many products causes a series of unpredictable interactions with ecosystems, due to the releases of free silver ions from these nanoparticles (Akter, Sikder, Rahman, Ullah, Hossain, Banik, Hosokawa, Saito & Kurasaki 2018). These free Ag⁺ ions may cause cytotoxic, and/or genotoxic effects in living organisms, due to their high surface area and volume ratio. These negative effects of AgNPs mostly depend on the shape, size and other surface properties of nanoparticles and also the synthesis methods.

The adverse effects of AgNPs can be determined using different living agents, such as plants, algae, and some bacteria (Kumar, Pandey, Singh, Shanker & Dhawan, 2011; Dalai, Pakrashi, Nirmala, Chaudhri, Chandrasekaran, Mandal & Mukherjee, 2013; Kim, Klaine, Cho, Kim & Kim, 2010). However, plants are the most used agents to determine the effects of NPs. Among these plants, wheat is one of the most popular plants due to being commonly cultivated crop plants worldwide. There is much research that investigated the effects of different nanoparticles on wheat seed germination, seedling and/or antioxidative enzyme response (Doğaroğlu & Köleli 2017; Li, He, Xie, Wang, Bose, Sun, Hu & Yin, 2019; Du, Yang, Peng, Liang & Mao, 2019). The first stage, in which plants are exposed to foreign substances, is germination. A healthy germination process means that healthy plants and yields. Thus, determination of the effects of NPs on seed germination is important. According to the United States Environmental Protection Agency (US EPA), the seed germination test is one of the valid methods for determining the phytotoxic effects of contaminants (Khot, Sankaran, Maja, Ehsani & Schuster, 2012).

Although the interaction of different nanoparticles and ecosystems is discussed widely in many studies, Ag NPs and ecosystem interactions are still not well known. Most of these studies are focused on the phytotoxicity of different nanoparticles in a short time exposure, such as seed germination, root-shoot elongation, and/or bioaccumulation processes (Yang, Jiang, Ma, Rui, Rui, Adeel, Cao & Xing, 2018). However, numerous studies in the literature focused on the antimicrobial activity of AgNPs, not growth parameters such as mentioned above (Pardha-Saradhi, Shabnam, Sharmila, Ganguli & Kim, 2018; Lee et al. 2012). Since the lack of knowledge about the effects of AgNPs on the early growth stage of plants, we reviewed the studies that investigated the effects of AgNPs on wheat germination.

MATERIALS AND METHODS

Search Strategy

This review organized according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement 2009 (Moher, Liberati, Tetzlaff & Altman, 2009), to evaluate the research articles about the effects of silver nanoparticles on wheat seed germination, and to help researchers in this area.

Four different electronic databases as ACS Publication, Science Direct, Springer Link, Taylor and Francis, were used to search related papers about the silver nanoparticles and wheat germination. The searching process was

conducted between the end of September and start of the October 2019. Different combinations of the keywords were searched as: “Silver nanoparticles AND wheat”, “silver nanoparticles AND seed germination”, “wheat AND seed germination”, and “Silver nanoparticles AND wheat AND seed germination”.

Study Selection and Data Extraction

The article selection in this study was dependent on two criteria. The first one is inclusion criteria that expressed as the publication year from 2009 to 2019; published articles in English; research articles; full-text available articles. The inclusion criteria were used to determine the title and the abstract of the articles. The second was exclusion criteria which expressed as not related to the inclusion criteria: incompatible title; the year of the article not between 2009 and 2019; different language from English, available only abstract; review and commentary article. All the searches in the database were reviewed independently by both authors. By the way, the references of captured articles were examined to determine if there was any missing relevant study, or not. Totally 7 articles were obtained after the evaluation of inclusion and exclusion criteria. They were critically assessed in matter the research methodology and results, in the discussion section in this study. This assessment is important to minimize the bias of individual results of studies.

RESULTS AND DISCUSSION

In this study, 52704 records were obtained from four different electronic databases, and among these records, 17251 were the incompatible titles and 35446 records were excluded according to the inclusion criteria. Thus, 27 articles were obtained but when they were examined, it was observed that 20 articles were not suitable for our inclusion criteria. The remained 7 articles were examined after selection and shown in Figure 1 as a flowchart. Fig. 1 presents the approach to determine according to inclusion and exclusion criteria with the number of articles considered at each step.

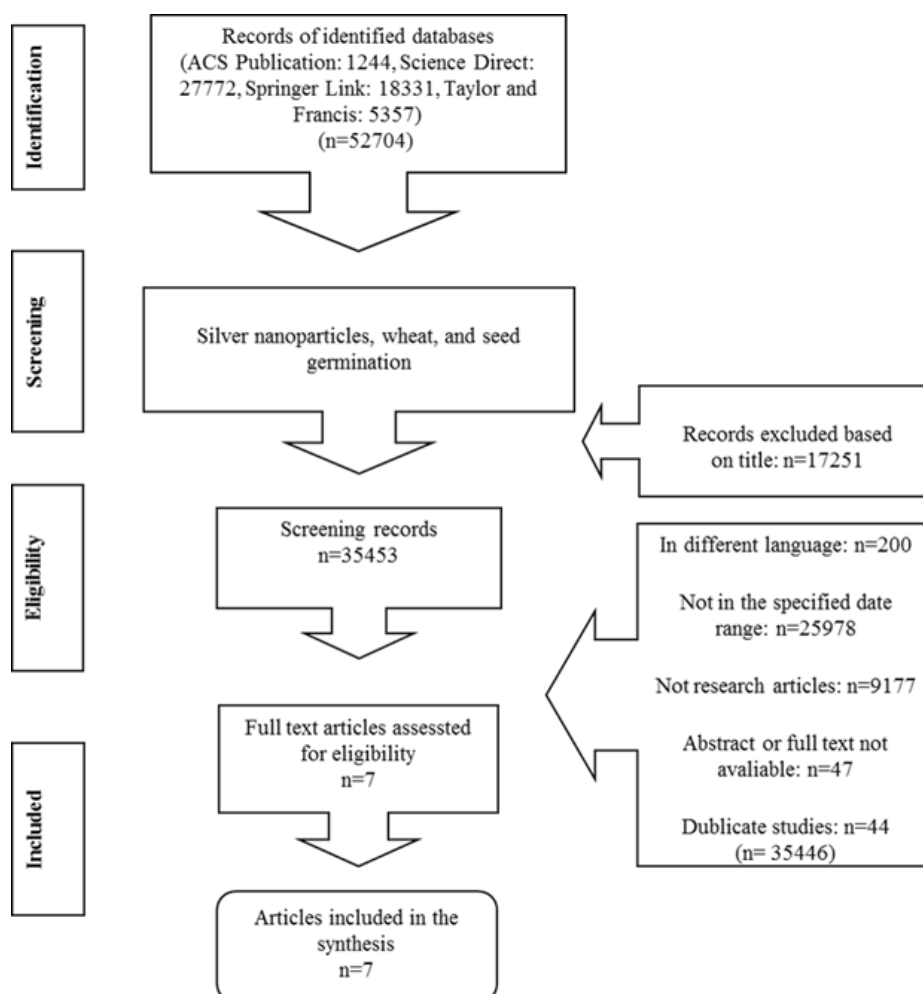


Figure 1. The Flowchart of This Systematic Review

In the included articles, the size ranges of the nanoparticles are higher than 10 nm and less than 100 nm, and also the treatment concentrations of AgNPs are varied between 23.8 µg/mL and 1200 mg/L. In these 7 articles, there is one article used only commercially purchased AgNPs. The other one is used only green synthesized AgNPs using different plant extract, while two of the remaining articles used only chemically synthesized AgNPs. By the way, in three of these seven articles investigated the comparison of the effects of green synthesized and chemically synthesized AgNPs or green synthesized and commercially purchased AgNPs on different wheat varieties (Table 1).

Synthesize Methods and Properties of AgNPs

In these 7 publications, authors have been examined the effects of four different AgNPs on wheat seed germination; (1) green synthesized AgNPs, (2) chemically synthesized AgNPs, (3) physically synthesized AgNPs and (4) commercially purchased AgNPs. Also, the characteristic properties (eg. size and shape) of synthesized or purchased AgNPs have been determined using transmission electron microscopy (TEM), high-resolution transmission electron microscopy (HR-TEM) or scanning electron microscopy (SEM). Determination methods for the morphological properties of nanoparticles using SEM and TEM are the most used techniques, and there are many studies in the literature about that (Jha and Prasad 2010; Cheng, Hung, Chen, Liu & Young, 2014; Srikar, Giri, Pal, Mishra & Upadhyay, 2016; Carbone, Paliotta, Micheli, Mazzuca, Cacciotti, Nocente, Ciampa & Dell'Abate, 2019). When the articles are examined depending on the size and shape of AgNPs in treatments, Vannini, Domingo, Onelli, De Mattia, Bruni, Marsoni and Bracale (2014) used commercially purchased PVP coating AgNPs in deionized water at the concentration of 1 and 10 mg/L. The authors determined the shape and size of nanoparticles using transmission electron microscopy (TEM). They noted the size of PVP-AgNPs as 10 nm, however, TEM analyses showed the mean size of nanoparticles as 13.2 nm. By the way, Kim, Saratale, Shinde, Syed, Ameen and Ghodake (2018) used both the biogenic AgNPs and commercially purchased AgNPs in their experiments. In this study, *Laminaria japonica* plants biomass powder used as the source of reducing agents for silver nitrate (AgNO₃) reduction. The authors determined the characteristic properties of synthesized AgNPs using TEM and they have mentioned the shape of synthesized nanoparticles determined as mostly spherical and few oval shapes. The commercially purchased nanoparticles have a spherical shape, also. The size of both used AgNPs types was mentioned as 20 nm. Another study investigated the effects of AgNPs (the average size was about 20 nm) on two different varieties of wheat (Kannaujia, Srivastava, Prasad, Singh & Pandey, 2019). The authors used four different Ag sources as biogenic AgNPs, chemically synthesized AgNPs, chemically synthesized AgNPs+10% fruit extract, and AgNO₃ solution. The biogenic AgNPs synthesized using *Phyllanthus emblica* L. fruit extract. High-resolution electron microscopy (HR-TEM) was used to determine the shape and size of biogenic AgNPs. Results showed that the shape of biogenic AgNPs was irregular but had a smooth edge. Different plant types and plant part extracts, such as leaves, whole plant biomass, root, shoot or fruit, can be used to green synthesis of AgNPs. The phenol content is the most important parameter in the selection of plants for researchers. The authors suggested that the synthesize standard of AgNPs can be constituted if the phenolic content of studied plants is given (Carbone et al. 2019). Carbone et al. (2019) used the white grape pomace extract which has high phenolic compounds for synthesized AgNPs, and they determined the effects of this nanoparticle on *Triticum turgidum* ssp. durum which, a kind of wheat. The authors determined the shape and size of green synthesized nanoparticles via scanning electron microscopy (SEM), and it was indicated that the AgNPs had dendritic shapes and >40 nm size. Singh, Kim, Zhang and Yang (2016) mentioned that the mechanism which plays a significant role in plant-based synthesized nanoparticles is not known well, because the different components and secondary metabolites are responsible for synthesizing different nanoparticles. Thus, different metallic nanoparticles can be synthesized from different plants, and this may affect the properties of the nanoparticles such as shape, and surface morphology. For this purpose, Amooaghaie, Saeri and Azizi (2015) synthesized and characterized the biogenic and chemically synthesized AgNPs, using *Nigella sativa* leaf extract and sodium borohydride, as reductant, respectively.

The authors determined the size and shape of both synthesized AgNPs using SEM, and results showed that the green synthesized AgNPs had 15 nm average size and mostly spherical shapes, while the chemically synthesized AgNPs had 30 nm average size and dominantly spherical shapes, too. By the way, the authors noted that the green synthesized AgNPs tend to small agglomeration while chemically synthesized AgNPs tend to large agglomeration. Differently from the green synthesize method of AgNPs, Gorczyca, Pocięcha, Kasproicz and Niemiec (2015) and Gorczyca, Przemieniecki, Kurowski and Oćwieja (2018) used physically and chemically synthesized AgNPs in their study, respectively. Gorczyca et al. (2015) synthesized the AgNPs via high voltage arcing method and

synthesized AgNPs characterized via TEM. The authors obtained colloidal AgNPs within the size range between 15 – 100 nm and the particles had spherical shapes. By the way, Gorczyca et al. (2018) used chemically synthesized AgNPs using tannic acid and AgNO₃. The authors determined the synthesized colloidal AgNPs within the size of about 16 nm and they did not mention the shape of particles.

Evaluation of The Selected Studies

The selected studies were evaluated according to US EPA and Organization for Economic Cooperation and Development (OECD) guidelines. Among these 7 articles, six articles sterilized the used seeds surface only Gorczyca et al. (2018) have not mentioned this step. The experiment's temperature changes between 20 and 25°C at room temperature. Applied concentrations are different and less than 100 mg/L in every study. The minimum applied concentration of AgNPs is 98.8 µg/L and the maximum concentration is 80 mg/L. The wheat seeds have been exposed to AgNPs for a minimum of 5 days and a maximum of 10 days. By the way, Gorczyca et al. 2015 have not mentioned the exposure time in their study. Most of these studies conducted in petri dishes. The seed germination test is usually conducted in petri dishes and the US EPA recommended this method in Ecological Effects Test Guidelines (1996). Many studies in the literature conducted the seed germination assay in Petri dishes with 5 mg/L test solutions (Lin and Xing 2007; Doğaroğlu and Köleli 2017). Seed germination and root-shoot elongation tests are the most used and recommended assays, because of cheap, simple and sensitive, to determine the phytotoxic effects of organic or inorganic chemicals.

Table 1. Studies on the Effects of AgNPs on Seed Germination of Wheat from 2009 to 2019

References	Green Synthesized (Gs)/Chemically Synthesized Commercially Purchased (Cp)	Particle Properties (Size and Shape)	The Concentration of Ag Nps	Variety of Wheat Seed	Experimental Condition	Germination Period (Exposure Time)	Target Analysis	Main Results in The Article
Vannini et al 2014	CP AgNPs coated with PVP	13.2 nm	1, and 10 mg/L	<i>Triticum aestivum</i> L. cv Blasco	100 × 15 mm sterilized Petri dish and Fifteen seeds per dish, 25 ± 1°C	5 days	Seed germination, shoot and root growth, biomass accumulation, and DNA damage	AgNP treatments had no adverse effects on seed germination. The root and shoot elongation decreased at the concentration of 10 mg/L AgNPs
Kim et al 2018	GS using <i>L. japonica</i> with AgNO ₃ (biogenic AgNPs) and CP AgNPs	GS-20 nm spherical-to oval-shape and CP-20 nm spherical shape	10, 20, 30, 40, 60, and 80 mg/L	<i>Triticum aestivum</i>	hydroponic culture	5 days	Seed germination, shoot and root growth	Seed germination was not affected by biogenic and commercial AgNPs.
Kannaujia et al 2019	GS using <i>Phyllanthus emblica</i> L. Fruits with AgNO ₃ , CS AgNPs, CS AgNPs+10% fruit extract, and AgNO ₃	~20 nm, irregular shape with smooth edge morphology	5, 10, 25, 50 mg/L	<i>Triticum aestivum</i> L. var. 'HD-2967' and var. 'DBW-17'	100 × 15 mm sterilized Petri dish and five seeds per dish, 25 °C	6 days	Seed germination, the radicle and plumule length, and Seedling vigor index (SVI), Reactive oxygen species (ROS)	Seed germination percentage was not affected by B-AgNPs treatment; however, it was decreased in AgNO ₃ treatment. The effective concentration of B-AgNPs was 10 mg/L in seed germination, thus the authors evaluated the other seedling parameters depending on this concentration. All seed germination parameters were significantly decreased with the treatment of AgNO ₃ and C-AgNPs. The addition of fruit extract with commercially synthesized AgNPs significantly improved seed germination percentage.
Carbone et al 2019	GS using white grape pomace extract with	>40 nm, dendritic structures	23.8, 36.4, 73.9 and 98.8 µg/mL	<i>T. turgidum</i>	soaking the 50 seeds with 3 mL of the	6 days	Seed germination, antifungal activity	There was no adverse effect on wheat seed germination. This green nanostructure had

	AgNO ₃			ssp. durum	four	AgNP solutions, 1 h, 20 °C, 200 rpm constant shaking, seeds transferred into Petri dishes			low effective on <i>F. graminearum</i> and there was not found dose-reduction relationship.
Amooaghaie et al 2015	GS using <i>Nigella sativa</i> with AgNO ₃ and CS AgNPs with sodium boro-hydrate	GS AgNPs: 15 nm and spherical in shape along with some of angular, and CS AgNPs: 30 nm and predominately spherical in shape	100, 200, 400, 800, 1000 and 1200 mg/L	wheat	Fifty seeds in glass petri dishes, 10 ml of each	10 days	Seed germination, root and shoot length		Both these synthesized nanoparticles had negatively effect on seed germination. Also the phytotoxic effects of GS AgNPs lower than chemically synthesized AgNPs.
Gorczyca et al 2015	CS AgNPs produced with using high voltage	ranging from 15 to 100 nm and spherical shape	4 mg /L	<i>Triticum aestivum</i> L. cv. Tybalt	100 seeds in each polypropylene cuvette filled with 100 mL test suspension or water. Four different applications: Control, AgNPs, <i>Fusarium culmorum</i> (Fc), and AgNPs+Fc, 20 °C	unspecified	Seed germination, root and leaves length, leaf area and dry weight (DW) of seedlings, antioxidative enzymes		Fc and AgNPs+Fc application inhibited the germination, significantly.
Gorczyca et al 2018	CS AgNPs with tannic acid	16 ± 4 nm and the shape of NPs not specified	100 mg/L	spring wheat cv. Bombona	Sixty seeds were grown in plastic pots filled with 0.8 kg of soil	5 days	Seed germination, dry biomass, chlorophyll content		CS AgNPs had no adverse effects on seed germination.

The phytotoxicity test is usually performed following the US EPA (1996) and OECD guidelines. So, in this section, the experimental procedure and results of selected 7 articles reviewed according to these standards.

According to US EPA (1996), 10 different plant seeds (tomato, cucumber, lettuce, soybean, cabbage, oat, perennial rye-grass, common onion, carrot, and corn) are recommended for use to determine the phytotoxicity of test chemicals and thus ecological effects. However, if the plants are economically or ecologically important, the other species can be used for the determination of phytotoxicity (US EPA 1996). Wheat is the most common cultivated and used plants worldwide. For this reason, we estimate the authors chose wheat seed in their studies. Vannini et al. (2014) determined the AgNPs with coating PVP did not any significant effects on wheat seed germination. The authors treated the 15 seeds in every petri dish with 5 mL PVP-AgNPs at the concentration of 1 and 10 mg/L for 5 days in the dark at room temperature in three replicates. Fifteen seeds cause the high density in a petri dish, in the US EPA and OECD test procedure for seed germination, which are mentioned that should be avoided from unnecessary crowded in petri dishes. According to these guidelines, 5 to 10 seeds should be in a 15 cm petri dish for small size seeds such as wheat (US EPA 1996; OECD 2006). By the way, the selected concentrations are not sufficient to determine the toxic effects of the test chemical, according to guidelines. It should be the minimum of five different concentrations with increasing geometrically and in the maximum fourfold. Thus, in this study, the phytotoxic effects cannot be determined correctly. The duration of the experiment was determined as 5 days in this study. Although there is no day-limitation in this parameter, it should be 65% and 50% germination rates in control groups according to US EPA (1996) and OECD (2006) guidelines, respectively. The authors noted the germination percentage of wheat seeds as 90%, so the test duration is suitable for this study. Meanwhile, Kim et al. (2018) reported that only 5 mL AgNPs at different concentrations were added to the hydroponic culture. However, they did not specify how many seeds were used in germination experiments, and the final hydroponic culture volume. The wheat seeds germinated nearly 100% under biogenic and commercial AgNPs treatment. The test duration may suitable according to the standard procedures mentioned above, but there is a lack of information about the germination assay. There was no information about the application in the experimental section, although the authors indicated that there were not any significant time-depend results of the direct treatment in the result section. The authors treated the wheat seeds in 0, 5, 10, 25, 50 mg/L B-AgNPs, C-AgNPs, C-AgNPs+10% FE and AgNO₃ concentrations in pre-germination test and decided the main treatment concentration as 10 mg/L. This concentration was determined as the most effective concentration of B-AgNPs in radicle length, plumule length, seedling vigor index, relative root elongation, and germination index (Kannaujia et al. 2019). Thus, the authors compared the effects of other chemicals on two different wheat varieties (DBW-17 and HD-2967) at 10 mg/L. It was reported the B-AgNPs has not been any adverse effects on both DBW-17 and HD-2967 (100% germinated seeds) however, C-AgNPs, C-AgNPs+10%FE, and AgNO₃ were significantly reduced seed germination. The authors noted that the DBW-17 had the sensitivity to these chemicals compared to control. Kannaujia et al. (2019) conducted a suitable experimental duration to guidelines; they used 5 seeds in every petri dish containing filter paper as an inert material, 5 mL test solutions, and the samples stored 6 days 25°C in the dark. By the way, Carbone et al. (2019) have applied a different germination test procedure from the others. The authors started their germination experiments with pretreatment as soaking durum wheat seeds with 3 mL test chemicals during 1h at 200 rpm. After seed soaking, they were transferred to petri dishes contained filter paper which soaked with distillate water, the dishes incubated for 6 days. This application may be done to accelerate the germination period, to get better seed germination and seedling growth, or to eliminate the phytic acid in wheat seed, etc. However, the authors did not mention in the article why the seed priming or soaking was applied to the seeds. In the literature, there are many kinds of research about seed soaking and/or seed priming. A very large percentage of these researches applied this procedure using the deficient nutrient element to get better germination and seedling growth (Cakmak 2008; Chen, Cheng, Hu, Guo, Chen, Lin, Hu, Bellizzi, Lu, Wang, Wang, Chen & Wang, 2017; Cheema, ur Rehman, Kiran, Bashir & Wakeel, 2018). Thus, the treatment type can be accepted suitable, according to guidelines. The authors indicated that 95% of seed germination occurred after incubation. So, the test duration time is suitable according to the standard test procedure mentioned before. The durum wheat seed germination was not affected from white grape pomace aqueous extract based AgNPs at different concentrations (0, 23.8 µg/mL, 36.4 µg/mL, 73.9 µg/mL, and 98.8 µg/mL). The concentration has been increased by nearly geometrically and two-fold. Amooaghaie et al. (2015) used *Nigella sativa* leaf extract-based and also chemically synthesized AgNPs, in six different plant seed germination tests at different concentrations (0, 100, 200, 400, 800, 1000, and 1200 mg/L). The authors indicated that the seed germination rate of both kinds of wheat and the other five different plants was inhibited by both green and chemically synthesized AgNPs. The phytotoxicity test on seed germination was conducted with 10 mL at different concentrations of AgNPs on fifty seeds in every petri dish and the dishes incubated for 10 days in a culture room. According to the seed germination standard tests, mentioned before, the

fifty seeds cause crowds in a petri dish. This is not preferred. By the way, the concentration range has been chosen geometrically increased and two-fold range in the first five values, and then increased systematically not geometrically. Since toxic effects occur at all concentrations, this systematic increase can be neglected. Gorczyca et al. (2015) were used a different germination procedure, as Amooaghaie et al. (2015). The authors conducted the test with 100 seeds in a polypropylene container containing 4 mg Ag/L nanoparticles suspension in a final volume at 100 mL. Four different treatments were applied, including control groups, AgNPs, *Fusarium culmorum* (Fc), and AgNPs+Fc. The authors aimed to determine the effectiveness of AgNPs against the fungal disease of Fc, like pesticides. For that, the authors investigated the effects of AgNPs obtained via high voltage arcing method, and AgNPs+Fc, on seed germination, photosynthetic activity and antioxidant enzyme analyses in wheat. All treatments were performed four times. The samples were placed in a growth chamber under suitable conditions mentioned in US EPA Guidelines (2012a, b, and c). According to US EPA Guideline (2012c), each treatment should be contained a minimum of 10 seeds in a 6-inch container and it should be avoided overcrowding. Also, according to US EPA Guideline (2012a), to determine the pesticide effects on plants a single concentration can be used. The authors determined the AgNPs+Fc inhibited the wheat seed germination significantly. On the other hand, they noted that the only AgNPs treatment did not affect the germination adversely. Gorczyca et al. (2018) conducted the germination experiments in soil medium. The authors used 60 wheat seeds in 0.8 kg homogenous soil in pots. The pots were incubated in a growth chamber under a suitable day/night cycle and condition. The authors noted that the maximum water capacity of the soil was 40%. By the way, there was no information about the soil characteristics, such as soil texture, pH, organic carbon/organic matter content, or soil type (natural or synthetic). However, all these properties of soil should also be investigated and reported. It should also be reported if the pretreatment was applied or not, such as heat treatment or pasteurizing (OECD 2006; US EPA 2012b). In this study, the soils in pots were soaked with a single concentration as 100 mg/L AgNPs and AgNO₃ solutions. Using a single concentration in plant-rhizoplane microbiome test is suitable according to OECD Guideline (2006) because the aim of this paper was a preliminary test to determine the effects of AgNPs on plant-rhizoplane microbiome system. The obtained 68% germinated seeds in the control groups were acceptable according to OECD Guideline (2006) which stated the 50% emergence seedling in control plants. The authors determined that the AgNPs and AgNO₃ chemicals did not any significant effects on seed germination of wheat compared to control.

Other Analysis in Articles

In addition to seed germination tests, many different analyses were performed in these studies such as root and shoot elongation, Ag bioaccumulation in plants, cytotoxicity of AgNPs, chlorophyll content, and antioxidant and antioxidant enzymes analyses.

CONCLUSION

The toxicological effects of nanoparticles released in the environment have not been known clearly. It was realized that most of the commercially or chemically synthesized nanoparticles were toxic to the aquatic and terrestrial environments. For this reason, some scientists started to produce less toxic or non-toxic nanoparticles to the environment. It was discovered that the plants can be used as suitable raw materials to produce different nanoparticles. In this review, we examined the seven different articles which used plant-based silver nanoparticles in the wheat seed germination stage. According to these publications, the plant-based AgNPs did not significantly adverse effects on seed germination of wheat compared to chemically synthesized or commercially purchased AgNPs. In addition to this, it was determined that the effects of AgNPs mostly depend on wheat types besides that the concentration, size, and shape of AgNPs, and treatment time and route were effective parameters. When the fate of AgNPs in the environment and the impact on organisms should be further investigated, it can be form basis for regulations about the AgNPs.

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