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The Effects of Several Metal Nanoparticles on Seed Germination and Seedling Growth: A Meta-Analysis

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Abstract: Using the proper means to improve seed germination is of great significance in agriculture and forestry. Here, a meta-analysis was used to examine whether metal nanoparticle treatments have a specific effect on the seed germination and seedling growth of agricultural species. Using the Web of Science (1950–2021), PubMed (1950–2021), and Scopus (1950–2021) databases, a paper search was conducted using the following items ("nanoparticles" and "seed germination", "nanomaterials" and "seed germination") to filter the references in the title, abstract, and keywords of the published articles. The results indicated that nanoparticle (NP) treatments had a significantly positive effect on the final germination percentage (FGP), with a mean difference (MD) (that is, the overall effect) of 1.97 (0.96, 2.98) for the silver (Ag)-NP subgroup, 1.21 (0.34, 2.09) for the other-NP subgroup, 1.40 (0.88, 1.92) for the total based on the NP types, 1.47 (0.85, 2.09) for the "Concentrations: <50 mg/L" subgroup, and 1.40 (0.88, 1.92) for the total based on the NP concentrations. Similarly, root length (RL) was positively and significantly affected by NP treatment, with an MD (95% CI) of 0.92 (0.76, 1.09) for the zinc (Zn)-NP subgroup, 0.79 (0.65, 0.92) for the other-NP subgroup, 0.82 (0.72, 0.93) for the total based on the NP types, 0.90 (0.77, 1.04) for the "Concentrations: ≤50 mg/L" subgroup, 0.80 (0.60, 0.99) for the "Concentrations: >50 mg/L" subgroup, and 0.82 (0.72, 0.93) for the total based on the NP concentrations. However, there was no statistical correlation between the nanoparticle concentrations and shoot length (SL), due to the inclusion of zero in the 95% CI of the overall effect. Therefore, Ag-NPs could increase the FGP more than other-NPs, while Zn-NPs enhanced RL more. Moreover, NPs at lower concentrations could improve the FGP and RL of crop species to a larger extent than NPs at higher concentrations. This meta-analysis can provide a reference for the nanoparticle treatment technology utilization in agricultural and forest seeds.

Keywords: metal nanoparticles; final germination percentage; root length; shoot length; meta analysis

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1. Introduction

Currently, advancements in manufacturing have led to the fabrication of nanoparticles with various sizes and shapes in large quantities; as a result, scientific studies have been conducted that investigate the environmental risks and toxic effects of nanoparticles [1,2]. Nanoparticles (NPs), with a particle size less than 100 nm in at least one dimension, can modify the physicochemical properties of a material compared with the corresponding bulk material, such as the reduction ability and conductivity, allowing them to efficiently enhance catalysis and to adsorb and deliver substances of interest [3–5]. Due to the continuously increasing production and use of nanoparticles in a variety of instruments and goods, plants are more prone to exposure to nanoparticles, which may accumulate in the living organs and cells of plants through direct exposure, contaminated soil, or air pollution [6]. In plant science, the most commonly used NPs are metal-based NPs, such as silver, titanium, zinc, and gold NPs, which were selected by certain researchers to better understand their effects on plants; therefore, mainly metal-based NPs (hereafter "metal NPs") are discussed in this study. Moreover, it is well-known that the effects induced by these materials are determined by the NP-type, the plant species, and the growth media, which are inconsistent among the various studies [7]. The effects of metal NPs on plants remain unresolved.

The phytotoxicity of metal nanoparticles has been studied for the plant species in seed germination and root elongation tests with the goal of promoting their use for agricultural applications in recent years, and the germination percentage (GP) and seedling vigor index (SVI) calculated from the root length and the shoot length as indicators are commonly used in seed germination and root elongation studies, as they provide a good estimate of potential field performance [1,2,4,8]. Data from limited studies have reported both positive and negative effects of metal NPs on higher plants (mostly agricultural species), which are therefore mainly discussed in this study [4,9]. López-Moreno et al. (2010) showed that the germination of corn, tomato, and cucumber seeds was decreased significantly (approximately 30%, 30%, and 20%, respectively) by nanoceria at 2000 mg/L [10]. For root growth, cucumber and corn root elongations were improved by nanoceria, while alfalfa and tomato root elongations were inhibited. Moreover, nanoceria improved the shoot length in the four plant species at approximately all concentrations. Feizi et al. (2013) found that nanosized TiO_2 with low and intermediate concentrations increased germination indicators, such as the germination value, vigor index, and mean daily germination, of fennel (*Foeniculum vulgare* Mill) seeds [11]. Moreover, the results found by Kumar et al. (2013) indicated that GNP (gold nanoparticle) exposure at both 10 and 80 μg/mL concentrations has significantly enhanced the seed germination rate, vegetative growth, and free radical scavenging activity of *Arabidopsis thaliana* [12]. However, seed germination, emergence, and the lengths of plumules and the principal and seminal roots of maize (*Zea mays*), were significantly inhibited by ZnO-NPs and CuO-NPs [13]. In addition, seed priming utilizing different types of metal NPs has been proven to enhance the seed germination and seedling vigor of agricultural species, which may be because NP treatment stimulates several metabolic mechanisms associated with seed germination, including the upregulation of aquaporin genes, α-amylase activity, reactive oxygen species (ROS) production, and antioxidant systems [8,14].

Thus, understanding the phytotoxic behavior of the metal NPs is necessary before using them under field conditions [2]. There were some reviews and systematic assessments concerning the metal NP effects on crop growth, while few meta-analyses could be found. Meta-analysis, mainly used in medical science, is a useful approach to explore whether metal NPs can promote seedling growth or not [15,16]. Therefore, the aim of this study was to explore the effects of metal NPs on the final germination percentage (FGP), root length (RL), and shoot length (SL) of the agricultural species through a meta-analysis to provide a meaningful reference for the wider utilization of the metal NPs, such as with forest species.

2. Materials and Methods

2.1. Data Collection

The data search was carried out in the Web of Science (https://www.webofscience.com/wos/alldb/basic-search), PubMed (https://pubmed.ncbi.nlm.nih.gov), and Scopus (https://www.scopus.com/search/form.uri?display=basic#basic) databases and was concluded on 3 September 2021. First, the keywords "nanoparticles and seed germination" were used to search for the articles via the three databases, and data visualization using social network analysis was conducted by means of the information (all records and cited references) exported from the databases (Figure 1). Studies reflecting the relationship between nanoparticles and growth, germination, oxidative stress, and antioxidant enzymes are abundant, indicating that nanoparticles may affect seed germination and seedling growth. Then, based on the data visualization, the search keywords ("nanoparticles" and "seed germination", "nanomaterials" and "seed germination") were utilized to screen the references according to the title, abstract, and keywords of the articles published without language restriction and without excluding any botanical family [17]. A selection process was then carried out to eliminate duplicate articles and those with topics not related to the objective of the meta-analysis. The papers that passed the first filter were submitted to a full text review by establishing selection criteria that each one had to meet to be accepted into the meta-analysis. The inclusion criteria of studies were as follows: (1) the seeds other than the seedlings were treated with nanoparticles; (2) both the control group (seeds treated with water) and the experimental group (seeds treated with metal nanoparticles) with three or more replicates were included in each study; (3) final germination percentage, root length, and shoot length were used as germination response variables in each study; (4) the mean and standard deviation (SD) for each variable and number of seeds used in the treatments were provided; and (5) only metal nanoparticle-involved studies were retained.

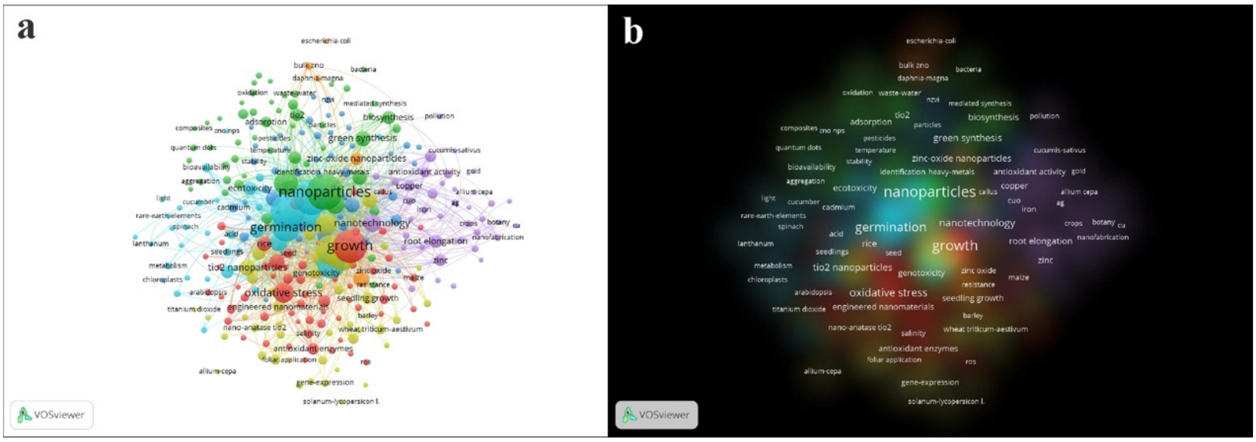

Figure 1. (**a**) Keywords of "nanoparticles/nanomaterials and seed germination" co-occurrence network visualization; (**b**) keywords of "nanoparticles/nanomaterials and seed germination" density visualization. The "curves" are the representatives of co-occurrence between the connected "nodes", and each node denotes a "keyword". Different keywords were segregated into different clusters, shown in different colors.

2.2. Risk of Bias and Publication Bias Assessment of the Included Studies

The qualitative aspects of the methodological process to evaluate the risk of bias for the included studies were as follows: sterilizing the seeds used in all treatments; seed germination and seedling growth under controlled conditions; seed exposure to a direct nanoparticle suspension; uniformity of the seeds; and cultivation of the seeds on the filter papers inside Petri dishes. The results were illustrated graphically in the forest plots of three indices, in which it was specified whether or not the inclusion criterion was met (a green color represents the study meeting the criterion with a low risk of bias, the yellow represents the study not meeting the criterion with an unclear risk of bias, and red represents the study not meeting the criterion with a high risk of bias). Publication bias was assessed through a funnel plot.

2.3. Germination and Seedling Growth Response Variables

We used the final germination percentage (FGP) (Equation (1)), root length (RL), and shoot length (SL) at optimal nanoparticle treatment concentrations as the main response variables for the germination treatments:

$$\mathrm{FGP} = S_g / S_s \times 100 \quad (1)$$

where FGP is the final germination percentage, S_g is the number of seeds germinated, and S_s is the number of seeds sown. The three outcome indicators were selected because they can reflect the effects of nanoparticle treatment on seed germination and seedling growth [8]. The outcome index values at the best concentration were selected, which can reflect the potential of nanoparticles to promote seed germination and seedling growth with little random error. Why not choose the value under the same concentration treatment or that under the same treatment time? The reason is that the variation in outcome indicators with nanoparticle concentrations does not show the same trend in each study. In addition, although the seeds used in each study belong to the crop species, there are differences in seed germination ability and germination time of different species or different varieties of the same species. Selecting these values would disturb the results of this meta-analysis and decrease its reliability.

2.4. Meta-Analysis and Statistical Analyses

The meta-analysis, an analytical method integrating the different results of various studies into a single common result, was developed through the international standard called Preferred Reporting Items for Systematic Meta-analyses (PRISMA) in this study [17]. In our study, Review Manager (Version 5.4.1) software was used to analyze the data for performing the meta-analysis to explore the effect of metal NP treatments on seed germination and seedling growth by calculating the mean difference (MD):

$$MD_{FGP} = T_{FGP} - C_{FGP} \quad (2)$$

$$MD_{RL} = T_{RL} - C_{RL} \quad (3)$$

$$MD_{SL} = T_{SL} - C_{SL} \quad (4)$$

where MD_{FGP}, T_{FGP}, and C_{FGP} represent the mean differences in FGP, FGP of the treatment group, and FGP of the control group, respectively. MD_{RL}, T_{RL}, and C_{RL} represent the mean differences of RL, RL of the treatment group, and RL of the control group, respectively, and MD_{SL}, T_{SL}, and C_{SL} represent the mean differences of SL, SL of the treatment group, and SL of the control group, respectively. Therefore, MD more than zero favors the treatment group. The chi-square (Q) test was selected to evaluate the study, subgroup, and total heterogeneity [18]. The inconsistency index (I^2) could be computed from the Q value and degree of freedom (df) according to Higgins et al. (2003) as follows [19]:

$$I^2\ (\%) = 100 \times [(Q - \mathrm{df})/Q] \quad (5)$$

A larger I^2 value indicated a high extent of heterogeneity or inconsistency among the data of the selected studies, and whenever the I^2 value was negative, it was set to zero, indicating no observed heterogeneity at all. In this meta-analysis, I^2 values lower than 25%, 25–50%, and above 50% represent low, moderate, and high heterogeneity, respectively [17]. All figures exported from Review Manager (Version 5.4.1) software in our study were processed using Adobe Photoshop software to obtain clear figures with proper sizes.

3. Results

3.1. Description of the Dataset

According to the social network analysis, the topics such as germination, root elongation, and growth included in this study were present in a large proportion. Then, by searching the databases, a total of 1860 articles were found, of which 884 were from the Web of Science, 271 were from the PubMed, and 705 were from the Scopus database. After removing the duplicates, screening for the title and the abstract, and selecting full-text articles with reliability and eligibility, and which met the inclusion criteria, 29 original articles were found to be eligible for the meta-analysis (Figure 2).

All of the included studies evaluated the effect of metal nanoparticle treatment on seed germination or seedling growth. Based on Table 1, all the species used by these studies belong to agricultural seeds, such as maize (*Zea mays*), radish (*Raphanus sativus*), barley (*Hordeum vulgare* L., cv. Annabell), rice (*Oryza sativa* L., cv. Swarna), and lettuce (*Lactuca sativa*), among others. There were various nanoparticle types, including Ag-NPs, Zn-NPs, Al-NPs, Ce-NPs, Fe-NPs, Cu-NPs, and Ti-NPs, with the exposure mode of suspension or suspension mixture with soil. The best concentrations of these metal NPs varied among different metal NP types, different indicators, and different species. In terms of the growth media, the seeds were mostly cultivated on filter papers in Petri dishes, followed by pots with soil, semisolid agar medium, and MS medium. Moreover, FGP, RL, and SL were evaluated as the outcome indices (Table 1).

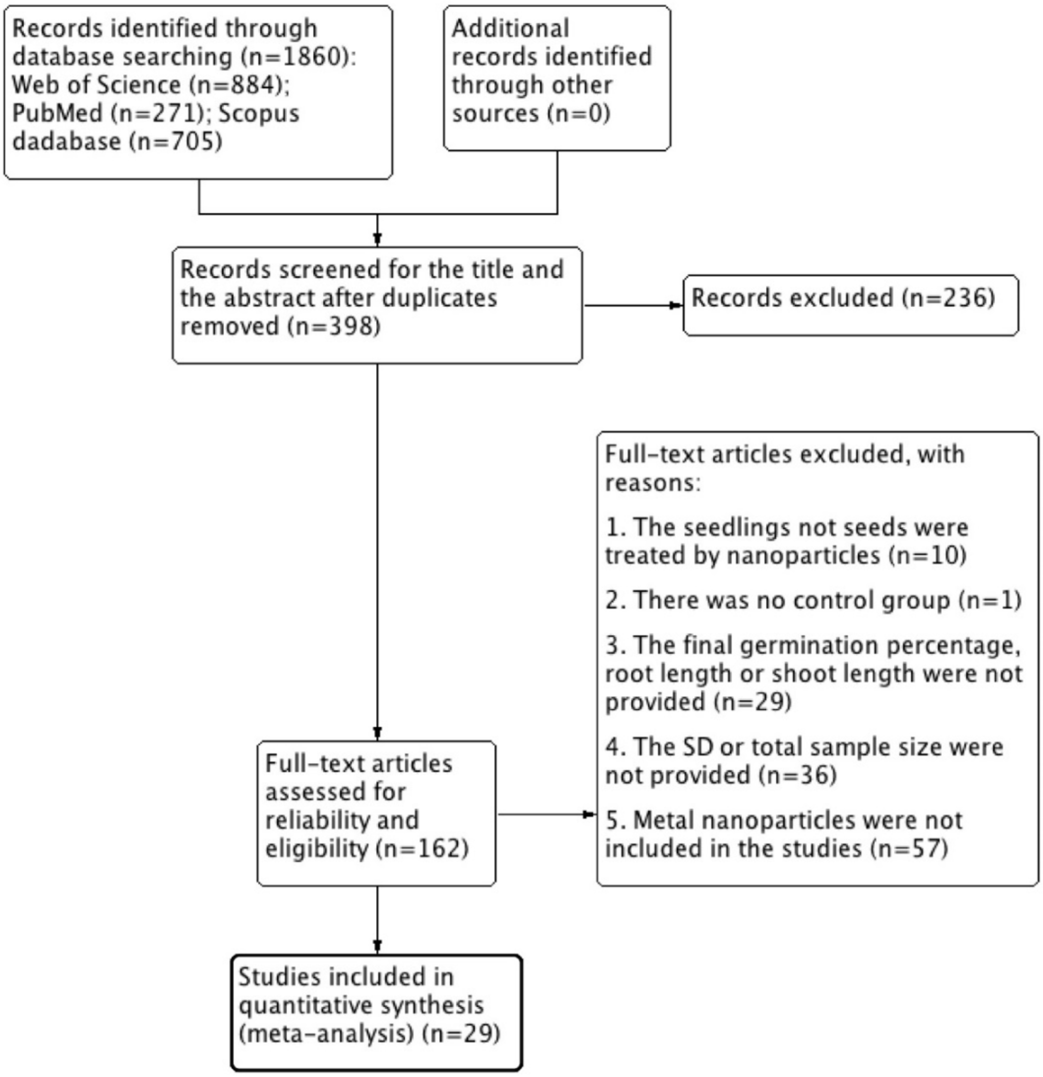

Figure 2. Flow chart of the study selection in the meta-analysis.

3.2. Risk of Bias and Publication Bias Assessment of the Included Studies

The funnel plot can be used to intuitively show the publication bias. The studies for the final germination percentage and root length analysis presented a roughly uniform distribution based on the overall effect lines (Figure 3a,b), while the studies for the shoot length analysis did not (Figure 3c). Among the three outcome indicators, studies with large sample sizes were in the majority, distributed at the top of the diagram, whereas those with small sample sizes were in the minority, accounting for fewer weight values in the heterogeneity analysis, which were distributed in the lower part. Overall, the studies for the shoot length analysis might exhibit a certain publication bias.

Table 1. Literature review of the studies included in the meta-analysis.

No.	Study	Seed Species	Metal Nanoparticle Treatment			Cultivation Media	Sample Size	Indicators
			Type	Exposure Mode	Best Concentration			
1	Acharya et al., 2020 [5]	Watermelons (*Citrullus lanatus*)	nano-Ag	Suspension	31.3 ppm	Filter papers in Petri dishes	400	FGP
2	Acharya et al., 2020a [20]	Onion (*Allium cepa*)	nano-Ag	Suspension	31.3 ppm	Filter papers in Petri dishes	300	FGP
3	Ahmed et al., 2021 [13]	Maize (*Zea mays*)	nano-Zn	Suspension	0.05 mg/mL	Semisolid agar in Petri dishes	90	SL
4	Belhamel et al., 2020 [21]	*P. vulgaris* var. "Piattelli"	nano-Al	Suspension	1.6 mg/mL	Filter papers in Petri dishes	50	RL
5	Corral-Diaz et al., 2014 [22]	Radish (*Raphanus sativus*)	nano-Ce	Mixture with soil	62.5 mg/kg [b], 125 mg/kg [c]	Pots with loamy sand soil	32	RL, SL
6	Duran et al., 2018 [23]	*Phaseolus vulgaris*	nano-Fe	Suspension	10 mg/L	Filter papers in Petri dishes	100	RL
7	El-Temsah and Joner, 2012 [9]	Barley (*Hordeum vulgare* L., cv. Annabell)	nano-Ag	Suspension	10 mg/L	Filter papers in Petri dishes	30	SL
8	Gupta et al., 2018 [24]	Rice (*Oryza sativa* L., cv. Swarna)	nano-Ag	Suspension	20 ppm	0.8% agar medium	30	RL
9	Kasote et al., 2019 [25]	Watermelon (*Citrullus lanatus*)	nano-Fe	Suspension	160 mg/L	Filter papers in Petri dishes	60	SL
10	Li et al., 2016 [26]	Corn (*Zea mays*)	nano-Fe	Suspension	50 mg/L	Filter papers in Petri dishes	30	FGP
11	Li et al., 2021 [27]	Fragrant rice varieties, Xiangyaxiangzhan and Yuxiangyouzhan (*Oryza sativa*)	nano-Zn	Suspension	50 mg/L [a], 25 mg/L [b]	Filter papers in Petri dishes	100 [a], 40 [b]	FGP, RL
12	Lin and Xing, 2007 [4]	Lettuce (*Lactuca sativa*)	nano-Al	Suspension	2000 mg/L	Filter papers in Petri dishes	30	FGP
13	Liu et al., 2018 [28]	Rice (*Oryza sativa japonica*)	nano-Cu	Mixture with soil	100 mg/L	Growth containers with soil	200	FGP
14	López-Moreno et al., 2017 [29]	Corn (*Zea mays*)	nano-Zn	Suspension	800 ppm	Filter papers in Petri dishes	30	RL
15	Mahakham et al., 2017 [14]	Jasmine rice (*Oryza sativa* L. cv. KDML105)	nano-Ag	Suspension	20 mg/L	Filter papers in Petri dishes	30	FGP

Table 1. *Cont.*

No.	Study	Seed Species	Metal Nanoparticle Treatment			Cultivation Media	Sample Size	Indicators
			Type	Exposure Mode	Best Concentration			
16	Nguyen et al., 2021 [30]	Green and red beans	nano-Zn	Suspension	10 mg/L [a,b,c]	Filter papers in Petri dishes	60	FGP, RL, SL
17	Saquib et al., 2016 [31]	Radish (*Raphanus sativus*)	nano-Fe	Suspension	0.25 mg/L	Filter papers in Petri dishes	60	RL
18	Segura et al., 2020 [32]	Radish (*Raphanus sativus*)	nano-Ag	Suspension	500 µg/mL	Filter papers in Petri dishes	30	RL
19	Singh et al., 2016 [33]	Tomato (*Solanum lycopersicum*)	nano-Zn	Suspension	1.2 mM [b,c]	Filter papers in Petri dishes	30	RL, SL
20	Singh et al., 2019 [34]	Wheat (*Triticum aestivum*)	nano-Zn	Suspension	250 mg/L [b], 15 mg/L [c]	Filter papers in Petri dishes	12	RL, SL
21	Singh et al., 2020 [35]	Wheat (*Triticum aestivum*)	nano-Ag	Suspension	25 mg/L	Filter papers in Petri dishes	75	FGP, RL, SL
22	Song et al., 2013 [36]	Tomato (*Lycopersicon esculentum*)	nano-Ag	Suspension	100 mg/kg	Filter papers in Petri dishes	50	FGP
23	Subpiramaniyam et al., 2021 [37]	Mung bean (*Vigna radiata*)	nano-Cu	Mixture with soil	1 mg/kg [b,c]	Glass beakers with soil	30	RL, SL
24	Sun et al., 2019 [38]	Mung bean (*Vigna radiata*)	nano-Fe	Suspension	450 mg/L [a], 150 mg/L [b]	Filter papers in Petri dishes	30	FGP, RL
25	Tan et al., 2017 [39]	Basil (*Ocimum basilicum*)	nano-Ti	Mixture with soil	750 mg/kg	Pots with topsoil	16	RL
26	Trujillo-Reyes et al., 2013 [40]	Radish (*Raphanus sativus*)	nano-Ce	Suspension	50 ppm [b,c]	Filter papers in Petri dishes	30	RL, SL
27	Wang et al., 2016 [41]	*Arabidopsis thaliana*	nano-Cu	Suspension	20 mg/L	MS media	60	FGP
28	Yadu et al., 2018 [42]	*Cajanus cajan*	nano-Ag	Suspension	1.2 nM	Filter papers in Petri dishes	350	FGP
29	Zuverza-Mena et al., 2016 [43]	Radish (*Raphanus sativus*)	nano-Ag	Suspension	125 mg/L	Filter papers in Petri dishes	120	FGP

Note: FGP—final germination percentage; RL—root length; SL—shoot length. [a] value for FGP; [b] value for root length; [c] value for shoot length.

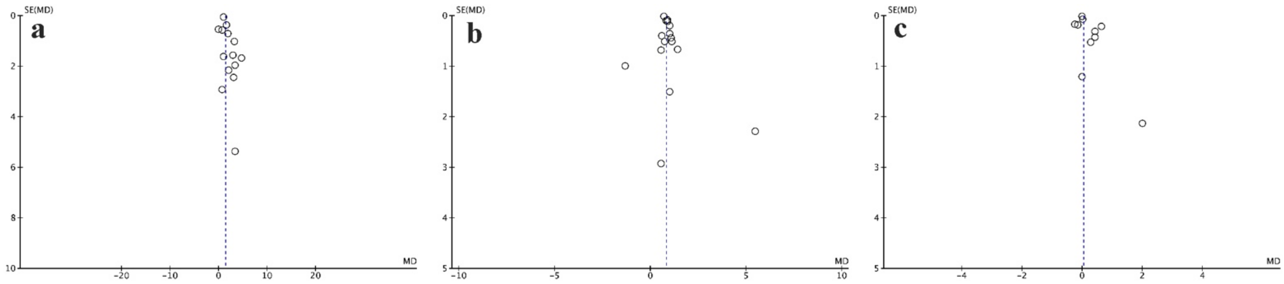

Figure 3. (**a**) Funnel plot for FGP; (**b**) RL; and (**c**) SL. The dotted lines represent the overall effects; hollow circles represent the studies.

The risk of bias assessment (Figure 4c, Figure 5c, Figure 6c) indicated that 64.29% of the studies for the final germination percentage analysis, 75% for the root length analysis, and 60% for the shoot length analysis performed the sterilization of the seeds used in all treatments, representing a low risk, whereas 35.71%, 6.25%, and 10%, respectively, did not illustrate whether the seeds were sterilized or not, representing an unclear risk; moreover, 18.75% and 30% for the latter two did not sterilize the seeds, thereby indicating a high risk. For the conditions utilized for seed germination and seedling growth, 71.43% of the studies for the final germination percentage, 62.5% for the root length, and 50% for the shoot length showed the detailed conditions, such as light intensity, photoperiod, temperature, etc., with a low risk, while 28.57%, 37.5%, and 50%, respectively, did not indicate the details, with an unclear risk. In 92.86% of the studies for the final germination percentage, 87.5% for the root length and 90% for the shoot length, the seeds were exposed to a direct nanoparticle suspension, showing a low risk, while in the remaining 7.14%, 12.5%, and 10% studies, respectively, the seeds were not, showing a high risk. All of the included studies for the three indicators reported the uniformity of the seeds with a low risk. The seed germination trial was carried out on filter papers inside Petri dishes in 85.71% of the studies for the final germination percentage, 75% for the root length, and 70% for the shoot length with a low risk, whereas 14.29%, 25%, and 30% of the studies had a high risk, respectively. Overall, 82.86% of the studies for the final germination percentage analysis, 80% for the root length analysis, and 74% for the shoot length analysis corresponded with all the evaluation criteria; however, 17.14%, 20%, and 26%, respectively, showed no accord with one or more criteria.

3.3. Effect of Metal Nanoparticles on the Final Germination Percentage

Based on the forest plot, a two-type subgroup analysis was carried out on the basis of nanoparticle types or nanoparticle concentrations to explore the effect of the two factors on the final germination percentage of seeds (Figure 4a,b). First, in the nanoparticle type-based subgroup analysis, two subgroups of Ag nanoparticles (Ag-NPs) and other-NPs were included, because other-NPs, such as Fe-NPs, Zn-NPs, Cu-NPs, and Al-NPs, involved studies in a few quantities, which would result in a decrease in meta-analysis reliability. There was no heterogeneity between the two subgroups (p = 0.27, I^2 = 19%), while moderate heterogeneity existed in each subgroup (I^2 = 36% for the Ag-NP subgroup; I^2 = 48% for the other-NP subgroup). The overall effects of the two subgroups were significant ($p < 0.001$), with a mean difference (MD) of 1.97 for the Ag-NP subgroup, 1.21 for the other-NP subgroup, and 1.40 for the total NP-type subgroups; the 95% CI was 0.96 and 2.98 for the Ag-NP subgroup, 0.34 and 2.09 for the other-NP subgroup, and 0.88 and 1.92 for the total NP-type subgroups (Figure 4a), showing a significant effect of nanoparticle type on the final germination percentage. After dividing the subgroups, the heterogeneity of each subgroup did not show significant variation compared with the total, indicating that the nanoparticle type might not have been the major source of heterogeneity in this research.

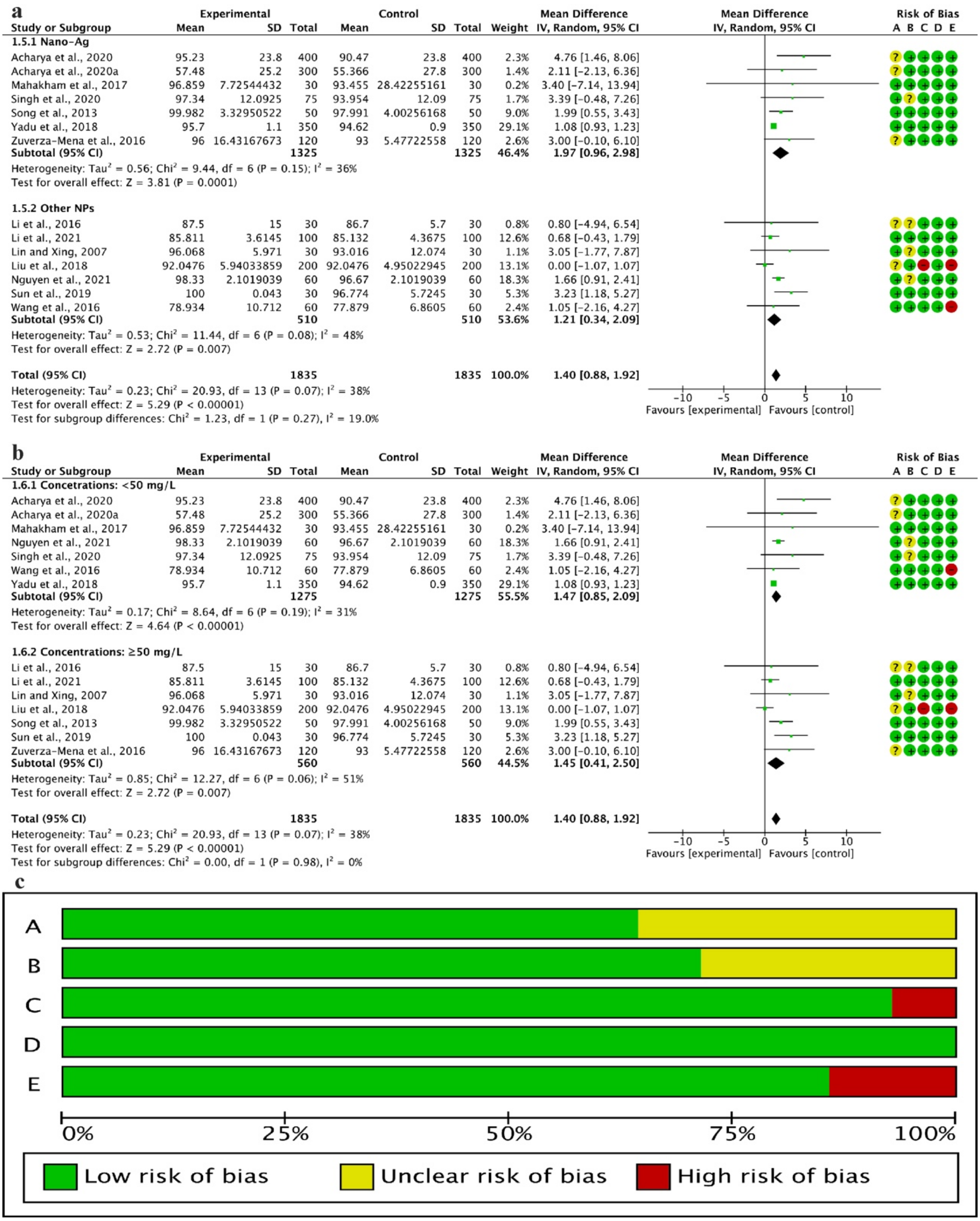

Figure 4. Heterogeneity evaluation, subgroup analysis, and risk of bias assessment for FGP. (**a**) Subgroup analysis for NP types; (**b**) subgroup analysis for NP concentrations; (**c**) risk of bias assessment, A—sterilizing the seeds used in all treatments, B—seed germination and seedling growth under controlled conditions, C—seed exposure to a direct nanoparticle suspension, D—uniformity of the seeds, E—cultivation of the seeds on the filter papers inside Petri dishes (the same below).

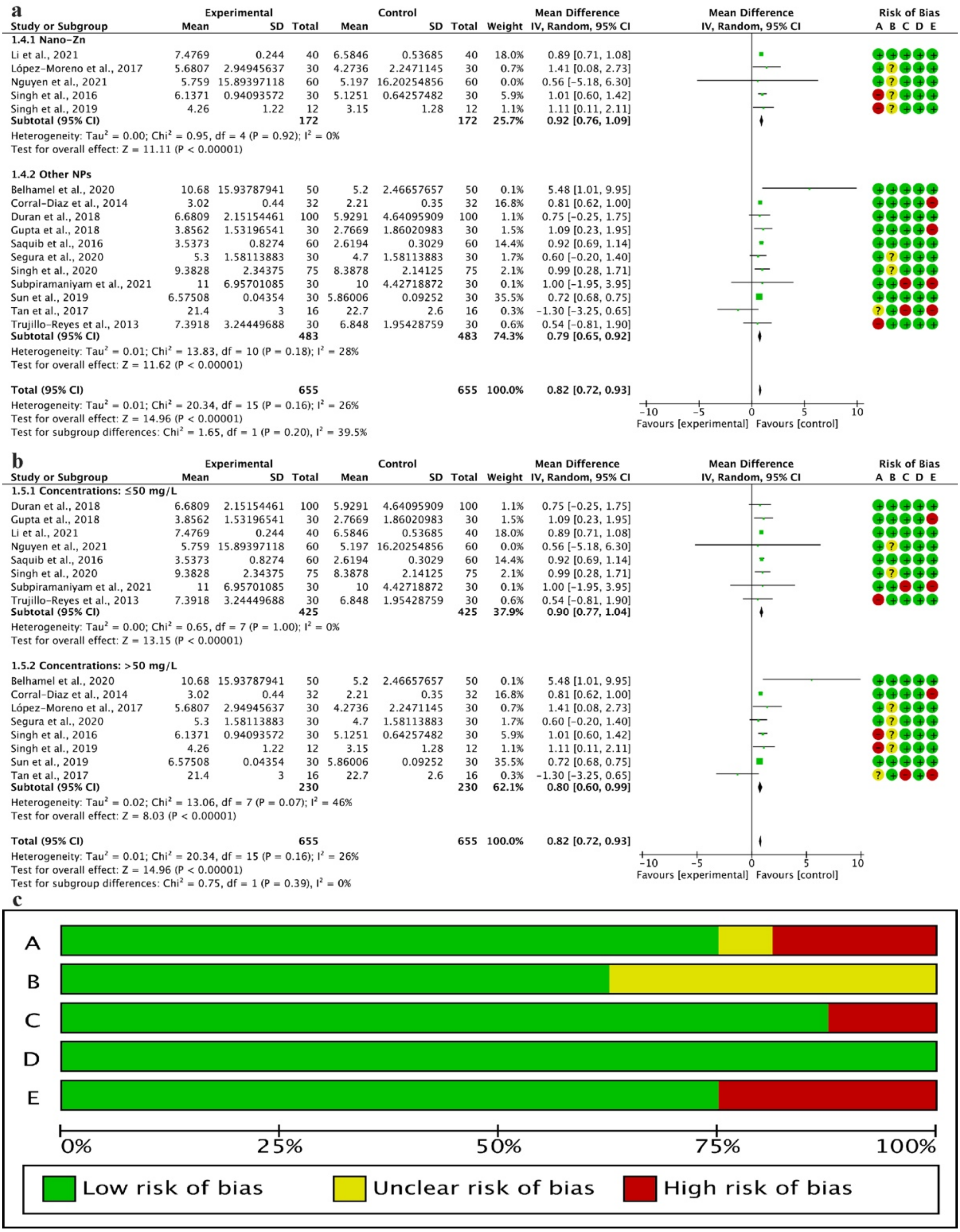

Figure 5. Heterogeneity evaluation, subgroup analysis, and risk of bias assessment for RL. (**a**) Subgroup analysis for NP types; (**b**) subgroup analysis for NP concentrations; (**c**) risk of bias assessment.

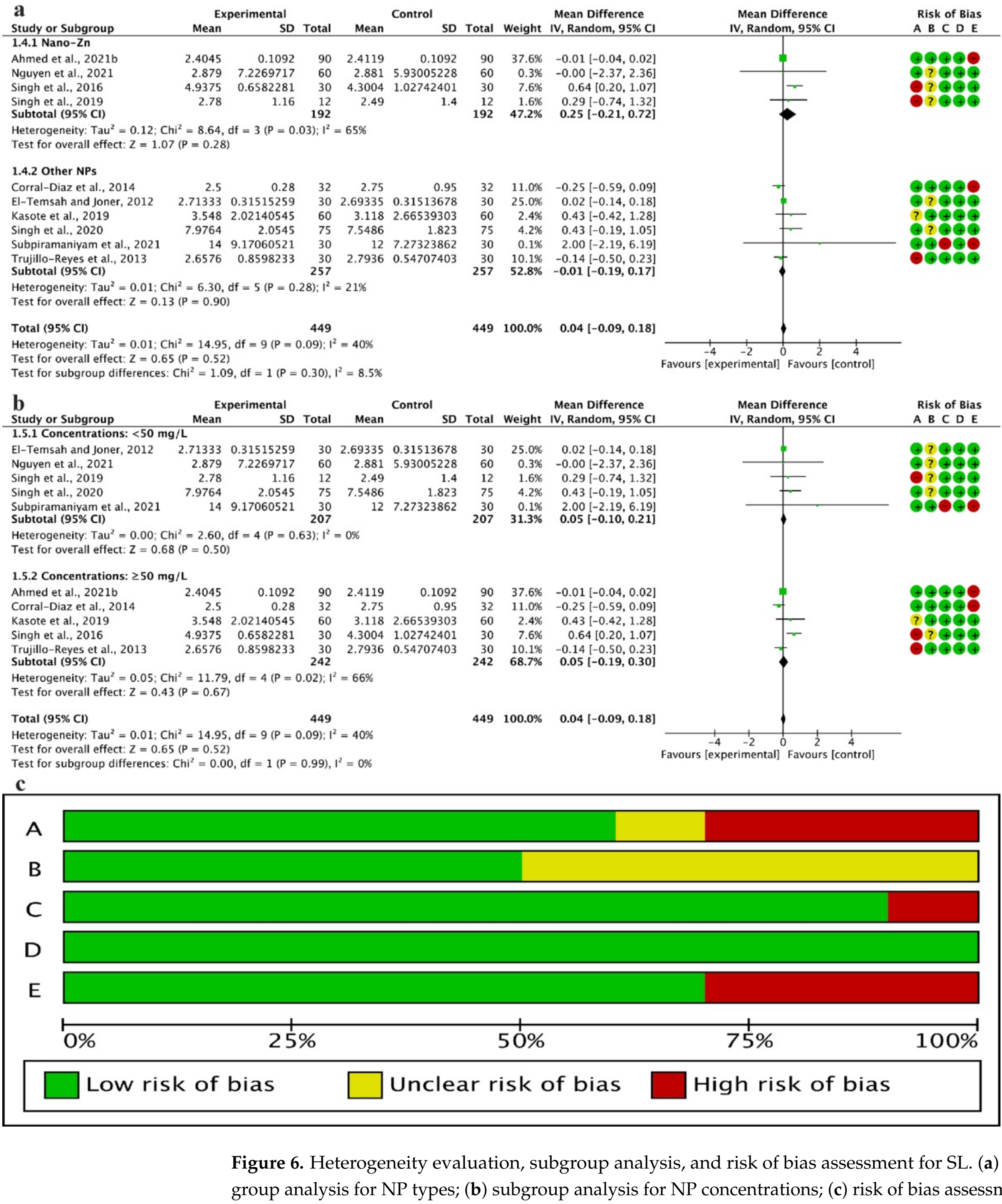

Figure 6. Heterogeneity evaluation, subgroup analysis, and risk of bias assessment for SL. (**a**) Subgroup analysis for NP types; (**b**) subgroup analysis for NP concentrations; (**c**) risk of bias assessment.

In the nanoparticle concentration-based subgroup analysis, there were two subgroups consisting of "Concentrations: <50 mg/L" and "Concentrations: ≥50 mg/L", for which approximately similar numbers of studies were included in each subgroup in this way. There was no heterogeneity for the two subgroups (p = 0.98, I^2 = 0%), whereas moderate heterogeneity existed in the "Concentrations: <50 mg/L" subgroup (I^2 = 31%) and the total (I^2 = 38%), and greater heterogeneity existed in the "Concentrations: ≥50 mg/L" subgroup (I^2 = 51%) with an unreliable overall effect. Therefore, the overall effects of the

"Concentrations: <50 mg/L" subgroup and the total were effective and reliable, with MD (95% CI) values of 1.47 (0.85, 2.09) and 1.40 (0.88, 1.92), respectively (Figure 4b), showing the significant effect of nanoparticle concentrations on the final germination percentage. Dividing the subgroups in this way did not markedly affect the heterogeneity in each subgroup compared with the total; as a result, the nanoparticle concentration may be not the major heterogeneity source herein.

3.4. Effect of Metal Nanoparticles on Root Length

Two-type subgroup analysis was also carried out based on the nanoparticle types (Figure 5a) or nanoparticle concentrations to explore the effect of the two factors on the root length of the seedlings (Figure 5b). In the nanoparticle type-based subgroup analysis, two subgroups of Zn-NPs and other-NPs were included, for which the reason was that other-NPs, such as Fe-NPs, Cu-NPs, Ag-NPs, Ce-NPs, etc., involved fewer than three studies, causing an unreliability of this meta-analysis. There was moderate heterogeneity between the two subgroups ($p = 0.20$, $I^2 = 39.5\%$), and for the other-NP subgroup ($p = 0.18$, $I^2 = 28\%$) and the total ($p = 0.16$, $I^2 = 26\%$), whereas no heterogeneity existed in the Zn-NP subgroup ($p = 0.92$, $I^2 = 0\%$), indicating that the nanoparticle types might be a major source of the heterogeneity. The overall effects of the two subgroups were significant ($p < 0.001$), with an MD of 0.92 for the Zn-NP subgroup, 0.79 for the other-NP subgroup, 0.82 for the total, and the 95% CIs of (0.76, 1.09), (0.65, 0.92), and (0.72, 0.93), respectively (Figure 5a), showing a significantly positive effect of nanoparticle types on the root length.

Additionally, in the nanoparticle concentration-based subgroup analysis, two subgroups consisting of "Concentrations: ≤50 mg/L" and "Concentrations: >50 mg/L", with approximately similar numbers of studies each, were included in this study. There was no heterogeneity between the two subgroups ($p = 0.39$, $I^2 = 0\%$) and for the "Concentrations: ≤50 mg/L" subgroup ($p = 1.00$, $I^2 = 0\%$), indicating the possibility of the concentration as the major heterogeneity source, whereas moderate heterogeneity existed in the "Concentrations: >50 mg/L" subgroup ($I^2 = 46\%$) and the total ($I^2 = 26\%$), showing that the concentration gradients of more than 50 mg/L may present different effects on root length, which requires more detailed division. Moreover, the overall effects were 0.90 (0.77, 1.04) for the "Concentrations: ≤50 mg/L" subgroup, 0.80 (0.60, 0.99) for the "Concentrations: >50 mg/L" subgroup, and 0.82 (0.72, 0.93) for the total, respectively, of the MD (95% CI) (Figure 5b), showing the significantly positive effect of nanoparticle concentrations on root length.

3.5. Effect of Metal Nanoparticles on Shoot Length

Similarly, two-type subgroup analysis was performed based on the nanoparticle types (Figure 6a) or nanoparticle concentrations to explore the effect of the two factors on the shoot length of the seedlings (Figure 6b). In terms of the nanoparticle type-based subgroup analysis, two subgroups of Zn-NPs and other-NPs were included, with a similar reason as the former two. Moderate heterogeneity existed in both subgroups ($p = 0.03$, $I^2 = 65\%$; $p = 0.28$, $I^2 = 21\%$) and the total ($p = 0.09$, $I^2 = 40\%$). The overall effects of the two subgroups and the total were not significant ($p > 0.05$), with an MD (95% CI) of 0.25 (−0.21, 0.72) for the Zn-NP subgroup, −0.01 (−0.19, 0.17) for the other-NP subgroup, and 0.04 (−0.09, 0.18) for the total (Figure 6a), which showed that there was no statistical correlation between the nanoparticle types and the shoot length due to the cross between the diamond (that is, the overall effect) and the invalid line.

In the nanoparticle concentration-based subgroup analysis, two subgroups consisting of "Concentrations: <50 mg/L" and "Concentrations: ≥50 mg/L", with approximately similar numbers of studies each, were included in this study. There was no heterogeneity for the "Concentrations: <50 mg/L" subgroup ($p = 0.63$, $I^2 = 0\%$), indicating the possibility of the concentration as the major heterogeneity source, while there was moderate heterogeneity for the "Concentrations: ≥50 mg/L" subgroup ($p = 0.02$, $I^2 = 66\%$) and the total ($p = 0.09$, $I^2 = 40\%$). The overall effects of subgroups and the total were ineffective because zero was included in the 95% CI, with an MD (95% CI) of 0.05 (−0.10, 0.21) for the "Concentrations: <50 mg/L" subgroup, 0.05 (−0.19, 0.30) for the "Concentrations: ≥50 mg/L" subgroup, and 0.04 (−0.09, 0.18) for the total (Figure 6b). Similarly, there was no statistical correlation between the nanoparticle concentrations and the shoot length due to the inclusion of zero in the 95% CI.

4. Discussion

4.1. FGP Increased More under Ag-NPs Treatment

NP types have a positively significant effect on FGP, with overall effect values (1.97 and 1.21 in each subgroup) and their confidence intervals greater than zero. It can be seen that Ag-NPs can increase the FGP more than other-NPs. The seed germination process culminates in the rupture of the seed coat and the emergence of the radicle, which enables direct contact with the NPs in the soil media and potentially impacts the development of the seed [37]. Ag-NP is a growing hot topic for researchers owing to its imperative physiochemical properties [42]. Mahakham et al. (2017) indicated that Ag-NPs can penetrate the seed coat and accelerate water uptake to promote seed germination and starch metabolism in rice; additionally, Ag-NP priming can upregulate the expression of aquaporin genes, thus facilitating water and H_2O_2 diffusion, and increased H_2O_2 may act as a signaling molecule for stimulating the germination process [14]. Acharya et al. (2020) found that the seeds treated with Ag-NPs can improve the seed germination of watermelons through an eco-friendly and sustainable nanotechnological approach because NPs have the advantage of being able to trigger certain metabolic processes (e.g., enhancing the levels of glucose and fructose, thus promoting glycometabolism) that are normally activated during the early phase of germination [5]. Moreover, Acharya et al. (2020a) found that NP treatments selectively modulated ZA and GABA levels in onion seed germination compared with the control, and then significantly affected germination inhibitors in onion seeds along with these germination stimulators [20].

Exogenous application of Ag-NPs can also alleviate the damage of adverse conditions to seed germination and enhance the stress resistance of plants. Yadu et al. (2018) showed that Ag-NP treatment promoted the germination percentage of *Cajanus cajan* under fluoride stress, and decreased reactive oxygen species (ROS) levels by suppressing the expression of the NADPH oxidase (NOX) gene and lipoxygenase (LOX) activity [42]. Due to their involvement in regulating ROS generation and its scavenging, Ag-NPs can affect antioxidant enzyme activity levels and gene expression patterns [24]. However, the chemical composition, particle size, shape, synthesis methods, and surface coating of Ag-NPs and their exposure form, and the plant species used, should be taken into consideration, due to their various effects on plants [34,44].

4.2. RL Enhanced More under Zn-NPs Treatment

Nutrient uptake improvement is an objective in crop breeding. The root system plays an essential role in nutrient and water acquisition, and its architecture is the spatial arrangement of roots (such as root length) that affects the capacity of plants to access nutrients [45,46]. Guo et al. (2017) found that root proliferation in response to external nitrate is a behavior which integrates local N availability and the systemic N status of the plant [46]. In this study, Zn-NPs could enhance the root length more than other-NPs. As an important transitional metal, zinc is the only metal present in all six classes of enzymes that acts as a cofactor for many essential enzymes of plants at below threshold levels, and a functional component for several transcription factors [13,47]. In eukaryotes, zinc is mainly dominated by 10% of zinc-binding proteins and 36% of zinc proteins, which are involved in gene expression. Thus, zinc should be used to regulate stress-related gene expression, especially in harsh environments [48]. Moreover, Zn is also an essential micronutrient that plays a vital role in the growth and yield of plants by maintaining cell membrane integrity and cell elongation, protein synthesis, and stress tolerance in plants [34].

Zn has potential to increase the biosynthesis of chlorophyll and carotenoids; enhance the contents of pigment, protein, and sugar; and thereby improve the photosynthetic capability of the species [33,49]. Photosynthesis, a fundamental process, plays a significant role in the growth, dry matter production, and yield of species [50]. Wu et al. (2020) also revealed that Zn is regarded as an important factor in plant photosynthesis; the chlorophyll concentration increased significantly with the application of ZnO NPs (10–50 mg/L) [51]. It has been reported that Zn nanofertilizers can improve the fruit yield and quality of pomegranate (*Punica granatum* cv. Ardestani) without affecting its physical characteristics, and ZnO-NPs could have been used as a fungicide in agriculture; thus, seed germination can be improved by treating the seeds with ZnO-NPs [29,30]. ZnO belongs to the class of metal oxides, which is characterized by photocatalytic and photo-oxidizing capacities against chemical and biological species [33]. Indeed, ZnO-NPs play a principal role in physiological and anatomical responses, as well as in hormone metabolism [30]. The root and shoot length of fragrant rice were substantially enhanced with ZnO-NP treatment, which induced modulations in physiological and biochemical attributes, e.g., the superoxide dismutase (SOD) activity, peroxidase (POD) activity, and metallothionein contents in roots, which were increased under low levels of ZnO-NPs [27]. In addition, ZnO-NPs can improve plant growth and induce resistance responses against *Sclerospora graminicola* in pearl millet by activating defense signaling pathways [48]. Notably, green ZnO-NPs could be used as a better material for agricultural products, such as nanofertilizers or nanopesticides, relative to their chemically synthesized counterparts [34].

4.3. FGP and RL Improved More in Lower Levels of NPs

In this study, NP concentrations also significantly affected FGP and RL, and the results indicated that NPs at lower concentrations could improve the FGP and RL of agricultural species to a larger extent. Several studies have obtained similar results; for example, the germination index of corn seeds under treatments with 20 and 50 mg/L γ-Fe_2O_3 NPs was 27.2% and 18.9% higher than that of the control, respectively [26]. Zuverza-Mena et al. (2016) showed that Ag-NPs increased the germination of radish seeds by 3% at the concentration of 125 mg/L, while they reduced the germination by 3% and 6% under 250 and 500 mg/L, respectively [43]. Therefore, the dosage represents a decisive factor influencing the ecological effects of NPs [38]. Higher Ag accumulation would change the structure of amino acids, nitrogenous bases, and nucleotides by forming complexes with them, interfering with the respiratory enzymes, and inducing oxidative stress in seeds, leading to a decrease in seed germination and seedling growth; in contrast, a low concentration of Ag-NPs with a short exposure time could not only reduce the toxicity of Ag-NPs but also enhance the germination and starch metabolism of aged rice seeds [14]. It is worth noting that the toxicity of NPs sometimes presents a nonlinear correlation with their dose, due to their aggregation with increasing density [38].

Similarly, the observation that NPs at lower concentrations can improve the RL of agricultural species to a larger extent has been evaluated in a variety of studies on crop species. Nanoparticle concentrations may affect the agronomic effectiveness of ZnO-NPs [33]. Under green ZnO-NPs at a moderate concentration (62 mg/L), the wheat seed samples presented the most significant enhancement ($p < 0.005$) in root length relative to other concentration levels by 50% [34]. In addition, ZnO-NP treatment at 50 ppm has been demonstrated to increase the seedling growth and reduce the excessive generation of ROS, while adverse effects on rice seedling growth have been observed at concentrations of 500 and 1000 ppm [27]. García-López et al. (2018) also found that ZnO-NP suspensions at 100, 200, and 500 ppm inhibited the seedling growth of *Capsicum annuum* and promoted the accumulation of phenolic compounds with phytotoxic effects [52]. Subpiramaniyam et al. (2021) found that the root length was slightly higher than that of the control (not significantly) under the lowest CuO-NP treatment (1 mg/kg) [37]. Moreover, γ-Fe_2O_3 NPs at a concentration of 20 mg/L significantly promoted root elongation by 11.5%, whereas those at 50 and 100 mg/L remarkably decreased root length by 13.5% and 12.5%, respectively [26]. Excess nZVI (zero-valent iron nanoparticles) accumulation in roots could not only block the uptake and transport of water but also disturb plant nutrient uptake and balance owing to the adherence of nZVI to the root surfaces and penetration into the root tissues, leading to reduced water flow and limited root hydraulic conductivity, thereby inhibiting the root elongation of the species [13,38].

However, there was no statistical correlation between the nanoparticle concentrations and the shoot length due to the cross between the diamond (that is, the overall effect) and the invalid line. Therefore, the variation trend of SL was more complex than that of FGP and RL in our study, possibly because in contrast to roots, which are likely to be most affected by NPs as the first organ to encounter soil-borne contaminants, shoots did not have direct contact with NPs, and the toxicity of NPs on SL depends not only on NP properties and environmental conditions but also on the test organisms and transportation capacity of the crop species [13,38,53]. Sun et al. (2019) illustrated that Fe-NPs accumulated in the roots are the nontransferable form with low mobility; thus, Fe-NP concentrations in shoots were not significantly affected [38]. However, there are similarities with FGP and RL, e.g., lower NP concentrations would promote shoot elongation more relative to the higher levels. The results of Li et al. (2021) indicated that the exogenous application of ZnO-NPs at a suitable concentration would be able to promote the growth of rice, whereas high levels could have inhibitory effects [27].

5. Conclusions

This meta-analysis presented the significant effects of nanoparticles on the final germination percentage and root length of crop species, based on the types and concentrations. Silver nanoparticles increased the final germination percentage more than other nanoparticles, while zinc nanoparticles enhanced the root length more. Moreover, nanoparticles at lower concentrations could improve the final germination percentage and root length of crop species to a larger extent than those at higher concentrations. The variation trend of the shoot length was more complex compared with the other two in our study, because the toxicity of nanoparticles on shoot length not only depended on nanoparticle properties and environmental conditions but also on the test organisms and transportation capacity of the crop species, and therefore, the heterogeneity, risk bias, and publication bias should be taken into consideration. In this global meta-analysis, nanoparticle effects on seed germination and seedling growth have been assessed mostly in agricultural species, whereas few studies have focused on tree seeds, which may show the more various dormancy types. One of the reasons why nanoparticles can promote seed germination and root growth is that they increase water permeability; moreover, the seed dormancy of some tree species is caused by difficulty in absorbing water. Therefore, nanoparticles may play a role in breaking this dormancy characteristic of certain tree seeds, indicating the application prospect of nanoparticle treatment technology in tree seeds in the future.

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