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Synthesis and Characterization of Zinc Nanoparticles by Natural Organic Compounds Extracted from Licorice Root and their Influence on Germination of *Sorghum bicolor* Seeds

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Abstract

This work aims to biosynthesize zinc nanoparticles (ZnNPs) from licorice (*Glycyrrhiza glabra*) root extract and to apply that on seeds germination of two *Sorghum bicolor* varieties (Enkath and Rabeh). Three concentrations of both licorice extract and colloidal solutions of ZnNPs (25%, 50%, and 75%) were tested on some features of planting seeds *in vitro* including Seeds germination percentage, shoot length, root length, seedlings length, and Root-Shoot Ratio. Along with that, characterization of ZnNPs was also done. AFM of the phyto-synthesized ZnNPs reached an average of 68.69 nm. Presenting of (-CH) group through FTIR confirmed finding compounds $–CH_3$ and $–CH_2$ in monosaccharides, disaccharides, polysaccharides and mono acidic saccharides. The exposure to ZnNPs showed remarkable effects on seed germination and other growth parameters of sorghum seedlings. The low concentration of 25% of ZnNPs exhibited the best shoot length compared with the high concentrations (50% and 75%). The two concentrations of 50% and 75% exhibited the presence of hairy roots in order to the smallness of roots. Thus, the low concentration (25%) of ZnNPs can be used as a material for *S. bicolor* seed priming in the field with low toxicity on this plant. The results of this work encourage using ZnNPs as an improver in agricultural applications.

Keywords: Agriculture, Biosynthesis, Licorice, Nanobiofertilizer, ZnNPs.

1. Introduction

Sorghum, *Sorghum bicolor* (L.) Moench, is a major crop of agriculture economically important cereals used as food for the humans in developing countries, and it is easily grown (Queiroz *et al.*, 2011). *S. bicolor* is produced in Iraq, and its total production was 50000 Million Ton in 2018 (IndexMundi, 2018). The world population is increasing rapidly, and the production is not sufficient to meet the requirements of the market. Thus, especially in developing countries, this can lead to severe challenges in food security (FAO, 2009). Sorghum ranks as the main cereal crop next to the wheat, rice and maize in the world (Soomro *et al.*, 2015).

Moreover, it has multiple usages and is included in the production of human and animal food, as well as alcohol and industrial products (Awika and Rooney, 2004). Cultivation of this crop is centered in tropical and subtropical regions, primarily in marginal areas that are more stress-prone (Reddy and Patil, 2015). These conditions of stress, especially abiotic ones, are the biggest causes of the reduction of sorghum yield (Souza *et al.*, 2015), although these are minimized owing to its higher tolerance to stress compared to other cereals (Mutisya *et al.*, 2009).

Micronutrients are chemical elements meaningful for plant growth. They are necessary at a small quantity and although their participation is small. The absence of essential micronutrients can lead to reducing the productivity of crops significantly (Malakouti, 2008). Micronutrient deficiencies are a common problem in soils of semi-arid regions, especially zinc, sulphur and boron (Sahrawat *et al.*, 2007). Thus, the reduction of the elements in the crop negatively affects human health (Tuomisto *et al.*, 2017). It has been assessed that many people throughout the world suffer from micronutrient malnutrition, the most common deficiencies of iron, zinc, and iodine, or vitamin A (Welch, 2002). Nowadays, nanomaterials priming of micronutrients is a new approach for the increase of seedling vigor and development of seed germination percentage (Dehkourdi and Mosavi, 2013; Ghafari and Razmjoo, 2013). Using nano-emulsion is significant to improve nutritional elements in plants called agricultural nanotechnology (Mostafa *et al.*, 2017).

Sorghum is the main cereal crop after wheat, rice and maize globally (Soomro *et al.*, 2015), and needs eco-friendly fertilizer to enhance its growth, and in this study, *Glycyrrhiza glabra* was selected to use in the biosynthesis of ZnNPs. *G. glabra* is one of the most traditional herbs used in medicine. It belongs to the family Leguminosae (Nomura *et al.*, 2002). However, *G. glabra* contains glycyrrhizin, liquiritin, liquiritin apioside, isoliquiritin,

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isoliquiritin apioside, liquiritigenin, isoliquiritigenin, licuraside, glycyrrhetinic acid, glycycoumarin, glabridin, licochalcone A licoricidin, *p*-hydroxybenzylmalonic acid (Li *et al.*, 2016), coumarins, flavonoids, triterpenoids, tannins, glabrol, kumatakenin, chalcones, licoricone, and phytosterols (Mostafa *et al.*, 2017). The root of *Glycyrrhiza* sp. is containing bioactivities such as antibacterial, antifungal, antimalarial, antiviral, anticancer, antioxidant, antiallergenic, immunostimulant, antiulcer, anti-inflammatory, antidiabetic, antithrombic, expectorant and estrogenic activities (Dong *et al.*, 2007; Renjie, 2008; Zore *et al.*, 2008; Al-Ani *et al.*, 2018).

There are many studies that have sought biosynthesis of metallic nanoparticles from organic compounds in plant (Al-Bahrani *et al.*, 2018) and fungi (Owaid and Ibraheem, 2017) such as greener zinc (Owaid *et al.*, 2019), silver (Owaid *et al.*, 2015, 2018) and gold nanoparticles (Owaid *et al.*, 2017). Green synthesis of ZnNPs is considered useful, safety/non-toxic and potential agent in different fields as has been produced from ginseng root extract (Owaid *et al.*, 2019). Hence, ZnNPs reduced the negative influence of drought action toward some plants (Taran *et al.*, 2017). Thus, in this study, licorice (*G. glabra*) root extract has been used to biosynthesize ZnNPs and tested their bioactivity in the agricultural field. This study aims to use ZnNPs synthesized using licorice extract for germination and feeding seeds of Sorghum (*S. bicolor*) *in vitro* in comparison with the aqueous extract of licorice alone. Seeds germination percentage, shoot length, root length, seedlings length, and Root-Shoot Ratio were investigated. Also, characteristics of the biosynthesized ZnNPs were studied.

2. Materials and Methods

2.1. Germination of Sorghum bicolor L. Moench Seeds

Two sorghum varieties Enkath and Rabeh (*Sorghum bicolor* L. Moench) were obtained from the Department of Yellow and White Maize Research, General Authority for Agricultural Research in Iraqi Ministry of Agriculture in May 2017 to use in the effect of the extracts and zinc nanoparticles (ZnNPs) on their growth *in vitro*.

2.2. Extraction of Glycyrrhiza glabra licorice root

Licorice root powder was obtained from the local market in Hit city, Iraq. About 5 g powder was extracted in 100 ml D.W (distilled water) by the magnetic stirrer hotplate for 15 min until boiling. The extracted solution was put for cooling then filtrated using gauze and centrifuged 4000 rpm. The aqueous extract has been collected and stored in the freezer until future use.

2.3. Biosynthesis of ZnNPs

For the licorice-mediated synthesis of ZnNPs, 10 ml of 3×10^{-3} M $ZnSO_4.7H_2O$ has been mixed with 3 ml aqueous extract of *Glycyrrhiza glabra* licorice root on the magnetic stirrer hotplate at 80 °C for 3 h until the change of color was seen.

2.4. Characterization of ZnNPs

The change of color, UV-Visible spectroscopy, FTIR (Fourier-transform infrared spectroscopy, Bruker), AFM (Atomic Force Microscopy), and Granularity Cumulation distribution have been achieved in Department of Chemistry at University of Baghdad (SPM AA300 Angstrom Advanced Inc., USA, AFM contact mode, with a suitable silicon tip by using IMAGER 4.31 software) to characterize ZnNPs formed using aqueous extract of *G. glabra* (licorice) root.

2.5. In vitro planting Sorghum bicolor seeds

Seeds were immersed in 5% sodium hypochlorite solution for 10 min to ensure surface sterility (U.S. Environmental Protection Agency, 1996). They were soaked in distilled water (DW) for 2 h, rinsed four times with DW, and then soaked in a series of the synthesized ZnNPs suspensions for approx. 2 h. Hence, ten seeds of *S. bicolor* were planted on the sterilized filter paper in 9 cm Petri dish and wetted by 5 ml of solutions separately for each concentration. All plates were put in the plant incubator at 25 °C for 10 days then harvested and the determinations were calculated. Seeds germination percentage, shoot length, root length, seedlings length, and Root-Shoot Ratio were recorded by using three concentrations of licorice root extract (25%, 50% and 75%) and three concentrations of colloidal solutions of ZnNPs (25%, 50% and 75%), while DW was used as control.

2.6. Statistical analysis

The data, in five replicates, has been subjected by its mean to Two-Way analysis of variance (ANOVA) using the SAS program, version 9. The significance of differences has been determined by using Duncan's Multiple Range Test, and the probability less than 0.05 was considered to be statistically significant.

3. Results and Discussion

The UV-Visible spectrum and the optical vision of zinc nanoparticles (ZnNPs) synthesized using the licorice extract were observed in Figure 1. The changing in the solution color from brown to the whitish bright brown (Pale yellow to pale white) recorded a lambda max reached 350 nm with the absorption of 4.200 au compared with the lambda max 350 nm for the extract which appeared because of the organic compounds at the absorption of 3.650 au. The results of the present study agree with results of (Tomaev *et al.*, 2019) who referred to the formation of ZnNPs in the range from 200-400 nm, and agrees with (Rajamanickam *et al.*, 2012) who synthesized polydispersed ZnNPs from Actinomycetes the lambda max reached 310 nm. The change of color is due to the excitement of surface Plasmon vibration in ZnNPs (Owaid *et al.*, 2019). The heating to 80 °C is more suitable than the low temperature for synthesizing ZnNPs from the extract of licorice roots due to increase of activation energy to reduce the organic biomolecules (Burda *et al.*, 2005).

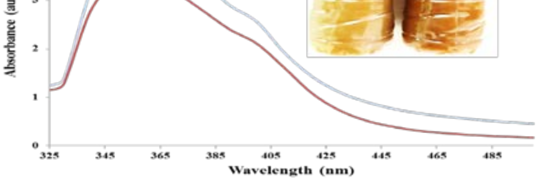


Figure 1. UV-Visible spectra and optical vision of the synthesized ZnNPs and the licorice extract .

Figure 2 exhibited AFM graphs (the three and lateral-dimensional graphs) to screen surface roughness of the licorice-assisted synthesis of ZnNPs at size image 1465.36 nm×1529.26 nm. The surface roughness analysis showed some functional parameters like roughness average of 2.92 nm, reduced core roughness depth of 10.3 nm, summit height of 0.91 nm, and reduced valley depth of 1.91 nm. Hybrid parameters were calculated such as surface area ratio reached 2.84, and mean summit curvature reached -1.0 nm^{-1}.

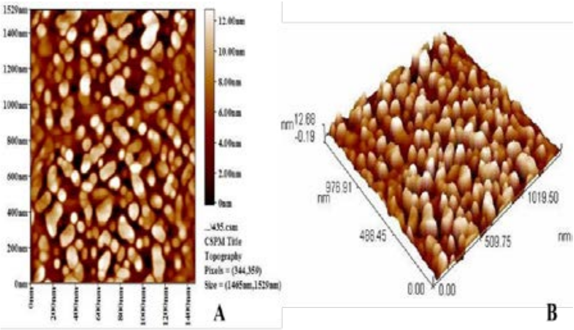
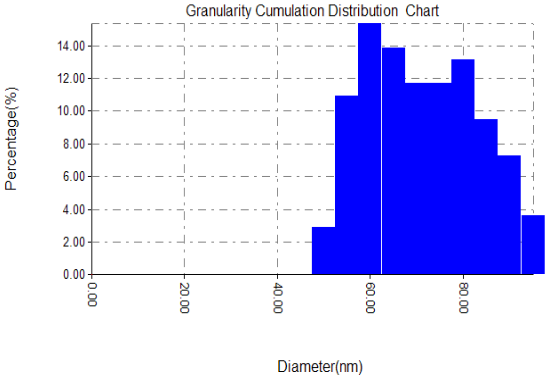


Figure 2. AFM of the biosynthesized ZnNPs, (A) 2D graph, (B) 3D graph

Figure 3 exhibited the histogram of the particle size distribution of the biosynthesized ZnNPs which reached to average 68.69 nm. The volume of ZnNPs of 65.00 nm was ≤50% as in the AFM. The lower ZnNPs diameter was 50 nm whereas the higher diameter was 95 nm with volumes 2.92% and 3.65%, respectively. The higher amount was 15.33% for the ZnNPs with a diameter of 60 nm. Granularity Cumulation distribution of ZnNPs also has different accumulation according to their sizes as shown in Figure 3. The ZnNPs of 50 nm have the lowest accumulation of 2.92%, followed 13.87% and 29.20% for ZnNPs with diameters 55.00 and 60.00 nm respectively. The higher accumulation percentage is 100% for ZnNPs of 95.00 nm, followed 96.35% and 89.05% for the sizes 90.00 nm and 85.00 nm respectively.

Figure 3 Granularity Cumulation Distribution chart of ZnNPs



Figures 4A and 4B showed FTIR spectra of the licorice extract and ZnNPs, respectively. These figures showed two bands at 1369 cm^{-1} and 1417 cm^{-1} for the extract and 1375 cm^{-1} and 1421 cm^{-1} for ZnNPs related to symmetric and asymmetric bending vibration for –CH group. Absorption bands at 2926 cm^{-1} for the extract and 2894 cm^{-1} and 2938 cm^{-1} for ZnNPs return to symmetric and asymmetric extension vibration for –CH group which refers to finding compounds of Alkane like $-CH_3$ (methyl) and $-CH_2$ (methylene) in monosaccharides, disaccharides, polysaccharides, proteins, amino acids and fatty acids. The presence of an absorption band evidence this at 1027 cm^{-1} for the extract and 1045 cm^{-1} for ZnNPs related to the single bond of C-C. The chemical composition of licorice may contain the protein, carbohydrates and fatty acids, and that agreed with the results of (Badr *et al.*, 2013).

Also, the spectra exhibited absorption bands at 1514 cm^{-1} for the extract, and at 1516 cm^{-1} for ZnNPs belong to extension vibration of C=C group for successive double bonds in the ring of aromatic compounds. On the other hand, the spectra exhibited absorption peaks at 3278 cm^{-1} for the extract and at 3277 cm^{-1} for ZnNPs return to the hydroxyl group (-OH) in amino acids and proteins (Silverstein *et al.*, 2005). Indications of the presence of fatty acids, amino acids and proteins are the presence of extension vibration of the broad absorption bands ranged from 2840 cm^{-1} to 3550 cm^{-1} for the extract and ranged from 2500-3500 cm^{-1} for ZnNPs which belong to the hydrogen bonding of the hydroxyl (O-H) in the carboxyl group (-COOH). In addition, the absorption band due to the bending vibration of the hydroxyl group (-OH) and the band located at 1199 cm^{-1} for the extract due to the extension vibration of C-O group (Silverstein *et al.*, 2005; Mistry, 2009). The presence of carbonyl group (C=O) in extension vibration at 1591 cm^{-1} for the extract and at 1597 cm^{-1} for ZnNPs and band of bending vibration at 831 cm^{-1} for the extract and the clear vibration at 866 cm^{-1} may belong to hydroxyl group in carboxylic acids (Hayashi and Sudo, 2009), and flavonoids (Kondo *et al.*, 2007). The sharp absorption band in Figure 4B at 1045 cm^{-1}, two bands at 1375 cm^{-1} and 1421 cm^{-1} are clear evidence for the presence ZnNPs in the sample.

The FTIR spectrum of the extract contains the ZnNPs; Figure 4B is similar in the sites of the functional groups. Also, ZnNPs are expected to have a large surface area that allows all atoms with high electronegativity which contains pairs of non-co-electrons like oxygen and nitrogen to contribute them well. For example, the oxygen atom in the composition of monosaccharides, oligosaccharides and polysaccharides in the form of –OH and in flavonoids in the form of ether (-O-) or in the form of carbonyl (C=O) and in the form of (-COOH). In amino acids, proteins and phenols are present in the form of (-OH); thus, the bond may be as follows: (Zn-O-R) where R is sugar, amino acids, or flavonoid. Another example of high electronegativity atoms is the nitrogen atom (N) in the amino acid composition ($-NH_2$) or (=NH) as in peptides. In protein, composition is in the form of ($-NH_2$); thus, it is likely that the bond may be as follows: (Zn-N-R) where R is an amino acid or protein. Finally, these organic compounds of the licorice extract reduced Zn ions to Zn atoms and covered them.

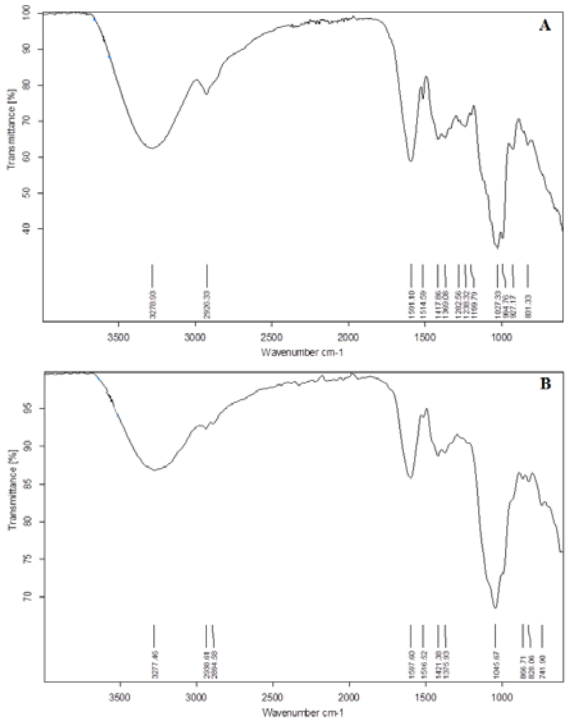


Figure 4 FTIR spectra of the licorice extract (A) and ZnNPs (B)

The germination percentage, shoot length, root length, seedlings length, and Root- Shoot Ratio of *Sorghum bicolor* seeds (Enkath and Rabeh varieties) were achieved *in vitro* for determination the influence of the licorice extract and the biosynthesized ZnNPs from licorice on the growth of *S. bicolor* seeds as in Table 1 and Figures 5 and 6. The germination percentage of Enkath seeds recorded germination percentage of 100%, while Rabeh showed 85% and 96% for the concentrations of extracts 25% and 50% respectively. The extract of 100%, and all concentrations of ZnNPs showed a percentage of seeds germination reached 100% as seen in Figure 5.

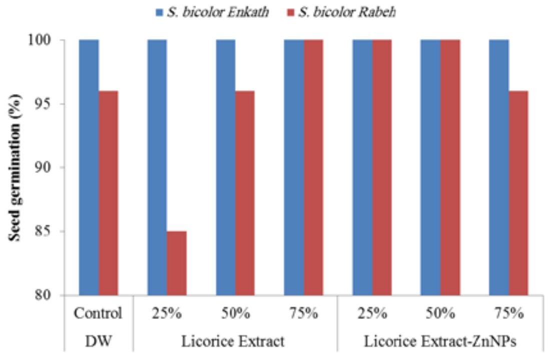


Figure 5 Percentages of Germination of *Sorghum bicolor* seeds

Rabeh variety significantly ($p<0.05$) recorded a high root length of 10.80 mm by using the extract of 50% compared to DW (Control) (9.06 mm), as shown in Table 1 and Figure 6. While the concentrations of 50% and 75% of ZnNPs led to root lengths reaching 3.14 mm and 5.10 mm respectively. Enkath showed a root length of 10.32 mm on DW followed by the extract of 25% (9.82 mm), while the shorter root was 5.18 mm on the 75% ZnNPs. Generally, shoot length was 5.43 mm by the extract of 50% while the ZnNPs (50%) decreased shoot length to 3.86 mm compared to 4.14 mm by DW. Rabeh recorded a more significant shoot length of 6.32 mm by the extract of 50% significantly ($p<0.05$) compared with 4.58 mm by DW, while the ZnNPs of 25% led to a shoot length of 3.74 mm.

In general, ZnNPs led to a decrease in the seedling length and root-shoot ratio of *Sorghum bicolor* plant *in vitro* compared with the licorice extract alone as in Figure 6. *Sorghum bicolor* Rabeh showed the best seedlings length in comparison with *S. bicolor* Enkath. Seedlings length of *S. bicolor* Rabeh has a more significant value 17.12 mm by the extract of 50% compared with 13.64 mm by DW, whereas the ZnNPs of 75% decreased the length of seedlings to 7.74 mm. *S. bicolor* Enkath recorded bigger seedlings length of 14.34 mm by the extract of 25% compared to 14.02 mm by DW, whereas ZnNPs of 50% decreased the length of seedlings to 9.62 mm. Generally, the Root-shoot ratio was 0.88 by ZnNPs (75%) but reached 2.03 by the extract (25%) as in Table 1. Hence, Figure 6 showed the increase in hairy roots in the higher concentrations of ZnNPs compared with the case of using licorice extract alone because of the increase of absorption mechanism in the hairy roots (Ghodake *et al*., 2010).

Table 1 Properties of *Sorghum bicolor* seeds growth *in vitro*

Features	Germination of seeds (%)			root length (mm)			shoot length (mm)			seedlings length (mm)			Root- Shoot Ratio		
Treatments	Enkath	Rabeh	Mean	Enkath	Rabeh	Mean	Enkath	Rabeh	Mean	Enkath	Rabeh	Mean	Enkath	Rabeh	Mean
DW 1	100a	96b	98b	10.32a	9.06b	9.69a	3.70d	4.58c	4.14cd	14.02b	13.64b	13.83b	2.90a	2.00bcd	2.45a
E25%2	100a	85c	92.5c	9.82ab	9.02b	9.42a	4.52c	4.96cb	4.74b	14.34b	13.98b	14.16b	2.20bc	1.86cd	2.03b
E50%3	100a	96b	98b	9.00b	10.80a	9.90a	4.54c	6.32a	5.43a	13.54b	17.12a	15.33a	2.02bcd	1.74cd	1.88b
E75%4	100a	100a	100a	6.76dc	7.52c	7.14b	3.66d	4.62c	4.14cd	10.42de	12.14c	11.28c	1.86cd	1.64d	1.75bc
N25%5	100a	100a	100a	6.02de	7.10dc	6.56bc	5.42b	3.74d	4.58bc	11.44cd	10.84de	11.14c	1.12e	1.94bcd	1.53c
N50%6	100a	100a	100a	6.74dc	5.10e	5.92c	2.88e	4.84bc	3.86d	9.62e	9.94e	9.78d	2.40b	1.04e	1.72bc
N75%7	100a	96b	98b	5.18e	3.14f	4.16d	4.76bc	4.60c	4.68bc	9.94e	7.74f	8.84e	1.08e	0.68e	0.88d
Means	100A	96.1B	98.07±0	7.69A	7.39A	7.54±0.81	4.21B	4.80A	4.51±0.56	11.90A	12.20A	12.05±0.89	1.94A	1.55B	1.74±0.35

Legend: Mean ±MSE (Mean Standard Error). The different small letters (a, b, c, etc.) show significant values ($p<0.05$) among each column, while the capital letters (A, B) exhibit a significant value ($p<0.05$) between means of Rabeh and Enkath in the row for each feature.

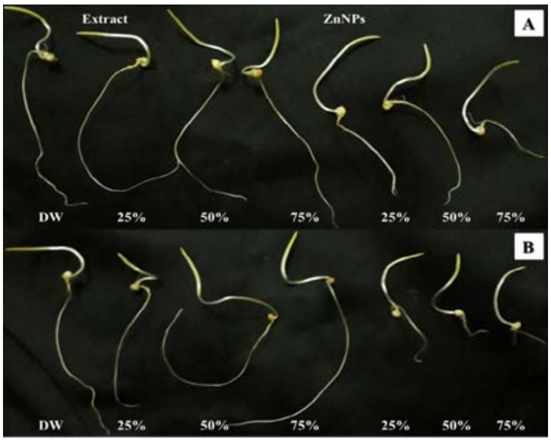


Figure 6. Planting *Sorghum bicolor* seeds (A) Enkath and (B) Rabeh *in vitro*

Table 2 exhibited significant ($p<0.01$) negative correlation (-0.846) between shoot length and seedling length, while the positive correlation (0.312) was recorded between root length and germination of *Sorghum bicolor* seeds. The higher results of the indexes of seedlings in sorghum in the case using licorice extract possibly return to the significant bioactivity of licorice related with glycyrrhizin (Fenwick *et al.*, 1990; Karkanis *et al.*, 2018). The results of the current study agree with the results of recent works (Burman *et al.*, 2013; Raskar and Laware, 2014; Zafar *et al.*, 2016; Raigond *et al.*, 2017).

The lower concentrations of zinc oxide nanoparticles (ZnONPs) increased Seed germination of onion (Raskar and Laware, 2014). The variety of Enkath was best than Rabeh in some features that related to genotypic characteristics of Enkath variety and agreed with leaf area less than Rabeh but with high weight (Wuhaib *et al.*, 2017). The lower concentrations of ZnNPs exhibited a significant increase in seed germination features of *Macrotyloma uniflorum* compared with the higher concentration (Gokak and Taranath, 2015). Also, ZnNPs had adverse effects on the indexes of seedlings in wheat (Taran *et al.*, 2017). The lower results of seedling in sorghum in the case of using ZnNPs may be related to ZnONPs exerting adverse effects on length of root (Burman *et al.*, 2013). The sorter seedling may have higher biomass in order to the harmful effects of ZnNPs in comparison with the extract alone in the high concentration (Burman *et al.*, 2013). In the case of use of ZnNPs, the higher activity of antioxidative enzymes stabilized the content of photosynthetic pigments and increased relative water content in leaves (Taran *et al.*, 2017).

Table 2 Correlation of properties of *Sorghum bicolor* Planting

	Root length	Shoot length	Root- Shoot Ratio	Seedlings length	Germination of seeds
Root length	1.000				
Shoot length	0.000	1.000			
Root- Shoot Ratio	0.000	0.000	1.000		
Seedlings length	-0.066	-0.846**	-0.062	1.000	
Germination of seeds	0.312	-0.074	0.005	0.084	1.000

4. Conclusion

This work aims to biosynthesize zinc nanoparticles (ZnNPs) from licorice (*Glycyrrhiza glabra*) root extract and to apply that on seeds germination of two *Sorghum bicolor* varieties, (Enkath and Rabeh). The AFM of the biosynthesized ZnNPs reached an average of 68.69 nm. The FTIR confirmed finding –CH group which refers to finding compounds $–CH_3$ and $–CH_2$ in monosaccharides, disaccharides, polysaccharides and mono acidic saccharides. The exposure to ZnNPs showed remarkable effects on seed germination and other growth parameters of sorghum seedlings. The low concentration of 25% of ZnNPs exhibited the best shoot length compared with the high concentrations (50% and 75%). The two concentrations of 50% and 75% exhibited the presence of hairy roots in order to the smallness of roots. Thus, the low concentration (25%) of ZnNPs can be used as a material for *S. bicolor* seed priming in the field with low toxicity on this plant. The results of this work are essential to determine the compatibility of ZnNPs in agricultural applications in cereals enhancement and food production.

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Conflict of Interest

None

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