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The effect of biosynthesis nano-zinc oxide on some growth indicaters of *Calotropid procera* L.

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Abstract
A laboratory -field experiment was conducted in one of the nurseries in the city of Diwaniyh for the agricultural Season 2021-2022 to study the effect of biologically prepared zinc nanoparticles. Nano zinc prepared from zinc acetate solution that Contains in molar ratios (0,1,2,3 mole) using extract of banana peels and raphanus root of the growth and germination of seedlings and some growth indicators, active ingredients of *calotropis procera*. completed the experiment with CRD design and three replications in a factorial organisation (2 x 4 x 3) and a LSD squared least parsing test was used in comparison. The averages are at probability level 5%.
The seeds planted in Petri dishes after soaking them in previously prepared solutions for 20 minutes. Then look measurements related to the seedlings after germination, and then the seedlings transferred to pots until reaching the vegetative growth stage.
The results showed that nano zinc particles for two types of extract had no effect on the germination rate, while they effected and gave in increase in the speed of germination of the shoot and root and length of each. Nano particles also increased the qualitative level extracted from the height of the Plant reached the high rate of 128.5cm. It also affected the number of lateral branches of the stems and also increased their concentration all of the active ingredients of the plant are &–tecopherol and Calotropin, significantly.

Keywords: Biosynthesis, nano-zinc oxide**,** *Calotropis procera*, environment, nano technology, green synthesis, plant extract

Introduction

Nano technology occupies a prominent place among the innovative methods for developing agricultural transactions and food production.
The wide variety of methods for preparing nano material Particles in research in the field of materials science, every, medicine and biology has provide clear facilitations for the use of nano technology and the expansion of the fields and applications in genetically modified plants and in techniques for producing fine agricultural chemicals (Narayanan and Sakthivel, 2011) [21]
The zinc nanoparticles are Particles whose size ranges between (l-100) nm and are characterized by their small. They behaive differently from traditional materials in the size of their Parties as well in their physical and chemical properties (Duhana, *et al*, 2017) [11], (Noor, *et al*, 2017) [24].
At the present time, the space of progress in nanotechnology has accelerated significantly, while recording several methods to preparing zinc nanoparticles. Including physical and chemical methods, methods of electrochemical and photo reduction methods and preparation by thermal evaporation (Das, *et al*, 2013) [9] and there has also been an increasing the manufacture of metallic Nano materials.Interest in natural resources because they provide functional diversity with the properties of the Nano materials (Zinc oxide) which is used to synthesize nanoparticles, and due the increasing need for these particles (AL-Ebadi, 2016) [6]. Synthesis processes must be developed, It is produced in environmentally friendly way through biological leaching, as it's less expensive and less toxic than other chemical and physical methods (Jeevanandam *et al*., 2018) [16]. Therefore researchers are looking to reach the safest methods for preparing nanoparticles. Among these methods, the safest and fastest, it is the use of extracts of leaves, seeds and fruits of plants (green synthesis), and others methods in preparing these particles to protect the environment.
The researcher (Diallo, *et al*, 2015) [10] prepared zinc nanoparticles from the leaves of plant *Aspalathus linearis* The annual extract from the leaves of the Rubeus plant, especially used by the researcher (Ambika and sundraya, 2015) [4] Phoenix herbal leaves trap these particles. Also The researchers (Liu, *et al*, 2020) and (Abdullah, *et al*, 2020) [1] prepared these particles from Raphanus roots and banana peels, respectively., and through these studies, it was proven that preparing these particles from aqueous extracts of different plant parts, it is environmentally friendly and safe in long run.
Based on the study conducted by the scientist (EL-Argawy, *et al*, 2017) [12] these particles have a role in improving plant growth, in addition to their role in resistance to agricultural diseases or pests. A number of researchers have conducted studies on the effect of nanoparticles on the growth and germination of plants seeds. The results of the researchers (Hojial, 2015) indicated that low concentrations of nanoparticles increased the germination and growth of fenugreek plants. Najafivafe, *et al*, 2015 explained stated that use of zinc nanoparticles at different concentrations on the thyme plant led to significant increase in plant growth indicators.
The *Calotropis procera* plant is one of the plants with great therapeutic Value and important economically and environmentally. It's a perennial, evergreen shuruby plant, that belongs to the Asclepiadaceae. stem height ranges between 2-15 m) spreads in tropical and sub tropic regions from Africa, which its original homeland of it (Moustafa and sararl, 2017) [20] its medical Importance came from the fact that it contains biologically active substances such as: glycosides, a, phenols, terpenes, and flavones, alkaloids, and others which are Considered an important source for

production medical drugs for various uses(Ranjan, *et al*, 2017) [26] it's considered an important drug for treating fever, rheumatism, Cough, eczema, asthma, painkiller, anti-tumor and anthelmintic. (Abed Al-Mageed, *et al*, 2016). It's economic importance lies in its use in the production of liquid biofuels, in the production of wood, and the fibers are used in the manufacture of ropes, Carpets and fishing nets (payal and sharma,2016).It's also considered one of the plants that combats environmental pollution (Galal, *et al*, 2016) [14].

Because of the economic, pharmacological and environmental importance of this plant, and since nanoparticles and zine oxidation especially, it play role in plant growth, this study aimed to green synthesize nano-zinc from natural materials instead of chemical fertilizers, that are safe for the environment at a lower cost and to study it's effect on medicinally used and environmentally important plants.

Materials and methods

A laboratory -field experiment was conducted in one of the nurseries in the city of Diwaniyah for the agricultural Season 2021-2022 to study the effect of biologically prepared zinc nanoparticles on growth and germination of seedlings of *Calotropis procera*. The plant extract of raphanus root and banana Peels, was prepared in the Nai Science and Technology Laboratory, Baghdad, and in the Preparation of nano zinc oxide from banana peels, the extract was prepared by adding 100 gm dried banana peel powder in the dark to 100 ml distil water only and it was heated for 20 minutes at Temperature 80c, and after refrigeration, the extract was filtered until obtaining the complete purity (Abdullal, *et al*, 2020). Later, zinc oxide particles were prepared by adding 50 ml from a solution of zinc acetate dehydrate and was added to the extract gradually with continuous Stirring for 10 minutes. The preparation process was repeated for three solutions of zinc acetate at a concentration of 1, 2, 3 molar, in addition to its concentration in the control treatment. In the previous method, nano zinc oxide was prepared from the raphanus roots plant according to the method used before Liu, *et al*, 2020. (Nanoparticles were characterized through scanning electron microscope SEM)

The nano zinc particles prepared from Raphanus roots extract were symbolized as R1, R2, R3 and banana peels symbolized as M1, M2, M3 and the number below the smart letter shows the molar concentration of zinc acetate used to form the Nano zinc particles, In addition to the control treatment and symbolizes it as Ro, Mo respectively.

To study the extent of the effect of the biologically prepared particles on the process of germination and growth of *Calotropis procera*. Anumber of seeds were soaked in different concentrations for a period time and 15 a minute, then put 5 seeds in each dish, and added 15 ml of distilled water to each dish and left the dishes in the laboratory, until seedlings germinate, after that measurements related to seedling growth were recorded:

The percentage of germination: was measured according to the following equation

$$\text{Germination percentage} = \frac{\text{number of seeds germinate}}{\text{Total number of seeds}} \text{ X100}$$

Germination speed (day) was calculated according to the method (Boras and Zidane, 2004) [7].

Germination speed= total number of germinate Seeds Per day X day number X number of germinate seeds at the end of the test Period.

And also the length of the plumule and radical of seedling was measured using a graduated ruler.

Then the seedlings were transferred to pots containing on a sandy soil, according to the table (1) (Nasseem *et al*., 2019).

Table 1: Physical and chemical characteristics of the field experiment soil

pH	E.C. ds.m-1	Organic matter %	N mg.kg-1	P mg.kg-1	K mm ch.L-1	Clay	Silt	Sand	Soil texture
						%			
.744	6.54	.110	90.50	38. 33	72.24	14.22	23.84	61.94	Sandy loam

At the end of vegetative growth period, measurements related to plant growth were taken, including the height of plant (cm), number of lateral branches of the plant and the content on the leaves in zinc and magnesium (microgram.gm.Dw), measured by an atomic absorption spectrometer and active substances in the plant were estimated using an HPLC device.

The results were analysed to a completely randomised design (CRD) and the results were tasted" by choosing the least significant difference LSD at the probability a level on 5% (AL-Rawi and Khalaf Allah, 1980) [5]

Results and discussion

Seedling growth indicators

Table 2 summarizes the results on the percentage and speed of germination for the seeds of *Calotropis procera* plant that were treated with different concentrations of bio-prepared Nano zinc particles for banana and Raphanus extract. The results obtained showed that the germination rate would not be affected by Kind and concentration of the extract. Likewise, the germination rate was not affected by the interaction between the concentrations and the two kinds of extract.

We notice from the same table the speed of germination increased with increasing concentration of the extract. The fastest germination rate was at 3 M concentration, which reached 3.2 days, with significant differences from the comparison treatment and 2 M concentration.

The speed of germination also increasing when using raphanus extract, reaching 3.3 days compared to 4.6 days when using banana extract with significant differences. the fastest germination was intraction between 1 molar concentration with the radish extract without significant differences from the rest of the banana extract concentrations.and this is comes back the result of cell division (Masoud and Bahurat, 2017), (farooq, 2005) [13]. The researcher (Kumari, 2011) [18] showed that the absorption of Nano-zinc through the roots can affect the rate of root germination and its length.

Table 2: Effect of zinc nanoparticles on the percentage and speed of germination seeds of *Calotropis procera*

Extract concentration	Percentage of germination % banana raphanus mean of ger			Speed of germination (day) Banana raphanus mean of sp.		
0	100	100	100	5.3	5.3	5.3
1	100	93	97	4.5	2.4	3.5
2	100	93	97	4.7	3.0	3.9
3	93	100	97	3.9	2.5	3.2
Mean of extract	98	97		4.6	3.3	
L.S.D 5%	N.S	N.S	N.S	0.46	0.33	0.65

N.S: Means no significant

It is noted from the table 3 that the banana extract increased the length of the plumule by percentage of 9.86% and without significant differences from the raphanus extract.

We also fined that the plumule length increases with Increasing concentration in the 3 molar concentration gave the highest length of 10.3cm, with a significant differences from the concentration.The best length of the feather was when interesting between a concentration of 3 molar and banana extract as it reached 11.4.

We also find that the bio-synthesized banana Nano extract encouraged an increase in radical length, reaching 8.5cm, with a similar increase of 49.12% with significant differences. Also, the concentration of 2 molar and 3 molar gave the highest length of the root, reaching 4.7cm and 9.1cm. Respectively, it was the Shortest length in the control treatment and was best when interacting between the banana extract and the 3 molar concentration, which gave the highest root length, reaching 11.9 cm with a significant difference from all the treatment, this agree with the researcher (Kumar,2011).

Table 3: Effect of zinc nanoparticles on the length of plumule and radical of seedling of *Calotropis procera*

Extract concentration	Length of plumule banana raphanus mean			Length of radical Banana raphanus mean		
0	3.4	3.4	3.4	4.3	4.3	4.3
1	5.4	6.6	6.0	7.1	3.3	5.2
2	10.3	9.9	10.1	10.1	8.8	9.7
3	11.9	8.6	10.3	11.9	6.3	9.1
Mean of extract	7.8	7.1		8.5	5.7	
L.S.D 5%	1.79	N.S	2.53	1.33	0.94	1.88

Growth characteristics

Table 4 shows and explains the results of plant height and the number of plant branches, which are among the vegetative growth characteristics of the plant. The plant height was shown when the plant was treated with Nano zinc Particles prepared from radish extract at a concentration 3 molar, but without significant differences with the rest of the treatments. As for the interaction between the type of extract and concentration. The highest plant height was observed at concentration 3 M with radish extract, it has reached 128.5 cm. The highest Plant height of 122.5 cm was observed at a concentration of 3 Molar with significant differences. As other type of extract, it did not significantly affect plant height.

The same table shows and summarizes the results of the number of Plant branches using the type of extract. The number of branches increased with the banana extract, reaching 3.20 branches. The Nano concentration also affected the number of branches of the plant, the largest number of branches was at a concentration of 3molar, as 3.63 branches were swallowed. The interaction also had a significant effect on the number of branches was when treated with banana extract with a concentration of & Moral with an increase rate of 89.15%.

The increase in vegetative growth of the plant was due to the contribution of zinc to plant growth, its effective contribution to the vital processes represented by the vital pathways of the meristematic cells (sharifi, 2016) [29] (Abou-shlell, *et al*, 2020) [3] It also has role in regulating sugar consumption and increasing energy, thus producing chlorophyll. And also because of his role in manufacturing auxins through its role in forming the amino acid tryptophan (Kadhim and athfua, 2023) [17]. This is agree with the results reached by the researchers Mendez, *et al*, 2016 treatment with nano zinc oxide led to an increase in vegetative growth of plant.

Table 4: Effect of zinc nanoparticles on the hight and number of branches of *Calotropis procera*

Extract concentration	Hight of plant banana raphanus mean			Number of branch Banana raphanus mean		
0	86.8	77.1	82.0	2.76	2.12	2.44
1	112.5	116.6	114.5	2.96	2.61	2.78
2	114.3	119.0	116.6	3.10	3.04	3.07
3	116.6	128.5	122.5	4.01	3.25	3.63
Mean of extract	107.55	110.30		3.20	2.75	
L.S.D 5%	N.S	N.S	4.93	0.305	0.193	0.193

According to Table 5 there was no significant effect of the treatments on the zinc and magnesium Content of plant leaves.

Table 5: Effect of zinc nanoparticles on the zinc and magnesium content of leaves of *Calotropis procera*

Extract concentration	Zinc content of leaves banana raphanus mean			Magnesium content of leaves Banana raphanus mean		
0	22.92	23.01	22.96	3.87	3.92	3.89
1	22.87	23.01	22.94	3.82	3.85	3.83
2	24.82	24.86	24.84	3.25	3.27	3.26
3	24.75	24.74	24.75	3.23	3.27	3.25
Mean of extract	23.84	23.90		3.54	3.57	
L.S.D 5%	N.S	N.S	N.S	N.S	N.S	N.S

Tables 6 shows the effect of Zinc nanoparticles on the percentage of active substances in the plant, it was observed that an increase in the substance calotropin in the plant leaves with increased the concentration. it was also noted that interaction between the factors had a significant effect in increasing the active substance in the leaves when treated with 3 Molar and banana extract as they reached 8.3 and 8.16 respectively compared to the control treatment

As for &-tecopherol substance, it was noted in the same table that it also showed that the substance increased significantly with concentration and according to the quality on the extract, its highest value was at a concentration of 3molar with the radish extract reached 5.15 %, the increase in the active substances under study is due to the positive effect in encouraging vegetative growth and thus increasing the efficiency of the plant, especially in the process of photosynthesis, it also participates in opening the stomata as it is acomponent of the carbonic anhydrase enzyme

necessary to maintain sufficient in the guard cells and affected the absorpation of potassium by the cells, thus leading to an increase in the active substance (chamani, *et al*, 2015) [8], also the biosynthesis of the materials under study requires the availability of zinc as acatalyst for the action of the enzymes that make up tyrosine, which increases with increasing zinc (Schenck, *et al*, 2017) [28].

Table 6: Effect of zinc nanoparticles on the percentage of active substances of *Calotropis procera*

Extract concentration	Caltropin banana raphanus mean			&-tecopherol Banana raphanus mean		
0	6.88	7.24	7.06	3.40	2.44	2.92
1	7.20	7.26	7.22	4.20	4.16	4.18
2	7.76	7.88	7.82	4.64	5.31	4.97
3	8.16	8.30	8.23	4.64	5.15	4.89
Mean of extract	7.50	7.67		4.22	4.26	
L.S.D 5%	0.010	0.019		0.36	0.68	0.48

Conclusion

The use of Nano- zinc oxide prepared from plant extract has had significant effect and increased plant growth indicators for both extracts.

Recommendation

We recommend using nanonutrients prepared in abiological way, especially from plant extract (green synthesis), instead of synthetic nanonutrient, as they are less expensive, safe and environmental friendly.

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