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Effect of Soaking Maize Seeds with Nano-Iron Fertilizer on Germination, Yield and Animal's Physiological Characteristics toxicity

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Abstract: A field experiment was conducted during the spring season of 2021 in the Experimental field of the College of agricultural engineering sciences - university of Baghdad with the aim of knowing the effect of soaking seeds with nano-iron at concentrations of (0, 50, 100 and 150 mg L^{-1}) on seed viability, field emergence, grain yield and components and grain Iron content The results showed significant increase in germination indicators, seedling field emergence, grain yield and its components accompanied by an increase of nano-iron concentrations up to 150 mg L^{-1}. Moreover, the results of seed Iron content showed accumulated of toxic level of Nano-Iron in seeds. The toxicity includes imbalance liver's functions, kidneys and oxidative imbalance of laboratory mice.

Keywords: Antioxidants, liver function, microelements, nanoparticles, seed stimulation.

Introduction

Maize (*Zea mays* L.) has great strategic value in Iraq and the world for its nutritional and economic importance due to its wide use in various food industries, so it ranks third globally after wheat and rice (Elsahookie *et al.,* 2021). However, the expansion of maize cultivation faces challenges such as limited land and water resources availability. To address these constraints, it is necessary to enhance the efficiency of natural resource use while preventing soil degradation caused by improper use of chemical fertilizers or reliance on traditional agricultural methods (Al-Rawi *et al.,* 2023). It is noted that the approved fertilization programs focus on macronutrients only without paying attention to micronutrients (Younis *et al.*, 2024). Among the most important of these nutrients is iron, which is an essential nutrient for all living organisms. It is essential in all physiological activities, including the process of chlorophyll synthesis in plants, oxidation and reduction reactions, DNA transcription, RNA synthesis, and auxin activity (Ali *et al.*, 2024). It is an element available in the soil, but the most abundant form in the soil is Fe^{+3}, which is an insoluble form, so it is not absorbed by the plant, as plants absorb iron in the form of Fe^{+2} (Al-Issawi & Al-Fahad, 2023a).

To get rid of the problem of weak nutrient absorption, the most important of which is iron in plants, we resorted to using Nano-fertilizer technology, which is one of the fastest advancing technologies in the world, which has proven highly efficient in its ability to penetrate plant tissues and interact at the molecular level within plant tissues (Al-Rawi *et al.,* 2024a). This technology has proven its worth in agricultural sciences through the production and application of environmentally friendly fertilizers, which are more efficient than traditional fertilizers (Jin *et al*., 2008; Alsulaiman & Al-Ansari, 2023). Bio-nanotechnologies can benefit many processes related to plant biology and improve Plant nutrition and addressing the increasing challenges in crop production, food security and seed protection from pathogens (Al-Rawi *et al.,* 2024b).

Iron nanoparticles fertilizer has proven its efficiency as a growth stimulant and increasing the plant's ability to absorb mineral elements from the soil in addition to increasing the chlorophyll content of leaves (Al-Rawi *et al*., 2024b), improving germination characteristics, vitality and activity of its seedlings and field establishment (Altayy & Cheyed, 2023), and increasing grain yield and its components (Mutlag *et al.,* 2023). The use of Iron nanoparticles fertilizers is one of the most effective and successful solutions in treating micronutrient deficiencies in developing countries by raising the nutritional content of field crops (Al-Shaheen *et al*., 2020).

From the above, studies have proven the efficiency of nano-iron fertilizers in improving crop growth, increasing field crop production, and raising their nutritional content without paying attention to the long-term physiological effects of these Nano-materials on the health of the plant itself and on the health of animals and humans when feeding on these plants or even when dealing directly with these fertilizers and the extent of nature's ability to get rid of nano-waste and prevent environmental pollution disasters.

Materials & Methods

A laboratory and field experiment were carried out during spring season of 2021 at Laboratory of Seed Technology and at Experimental Field, College of Agricultural Engineering Sciences - University of Baghdad. The aim was knowing the effect of nano-iron concentrations on the viability and vigor of seedlings and seed yield and its components of maize, Buhuth 106 variety, and the content of nano-iron in grains.

Laboratory experiment: A sample of maize seeds soaked in deferent Nano-iron concentrations (0, 50, 100 and 150 mg L^{-1}), for 24 hours, and the treatments were planted according to the Completely Randomized Design (CRD) with four repetitions, and the seeds number were 25 seeds per treatment.

Field experiment: The seeds were planted in rows with a distance between plants of 25 cm and a distance between row of 75 cm so the plant density of reached to 53333 plants ha^{-1}. An experiment was carried out with concentrations of 0, 50, 100 and 150 mg L^{-1}), according to the Randomized Complete Block Design (RCBD) with three replicates. The area of the experimental unit was 2×3 m² and 4 lines. The number of units was 12. A distance of 2 m was left between one sector and another and 1 m between one treatment and another to avoid interference. Nano-iron was prepared by dissolving 1 g in 1 liter of water, then the quantities of 50, 100 and 150 ml were withdrawn and the solution was completed to 1 liter to prepare the above concentrations. Distilled water was used as a comparison treatment. Samples of field soil were taken at a depth of 0-30 m. After mixing, grinding and removing impurities, they were sent to the central laboratory at the College of Agricultural Engineering Sciences - University of Baghdad to determine some physical and chemical properties of the soil (Table 1). The maize seeds were soaked in iron nanoparticles before planting.

Table (1): Physical and chemical properties of the experimental field soil before planting

Property		Unit	Value
Soil Reaction (1:1 pH)		-	7.11
Electrical Conductivity (1:1 EC)		dS m^{-1}	2.43
Soil Organic Matter (SOM)		g kg^{-1}	4.46
Gypsum (Ca SO_4)		%	0.89
Carbonate Minerals ($CaCO_3$)		%	13.4
Available Nitrogen (N)			8.93
Available Phosphorus (P)		mg kg^{-1} soil	21.3
Available Potassium (K)			13.8
	Soil Separates: Sand		19.1
Soil separates	Soil Separates: Silt	g kg^{-1} soil	42.1
	Soil Separates: Clay		38.8
Textural Class		Sandy Clay Loam	

Studied characters for the laboratory experiment:

Standard germination percentage (%): 25 seeds were placed between two layers of germination papers after soaking them for 24 hours with iron concentrations and control treatment and placed in the incubator at a temperature of 25 °C for 10 days.

Seedling vigor index: The seedling vigor index was calculated according to the following equation:

Seedling vigor index = germination percentage in the final count × [radical length (cm) + plumule length (cm)].

Studied characters for the field experiment:

Field emergence (%): The emergence percentage was extracted by dividing the number of emerging seedlings by the total number of seeds planted in the field after 11 days of planting.

Number of ear grains (grain $plant^{-1}$): After the seeds were separated, they were placed in a seed counter and the average number of seeds per ear was extracted .

Weight of 500 grains (g): 500 grains were taken from each experimental unit after loosening the cobs and mixing them, then weighing them on the scale.

Total grain yield (t h^{-1}): Extracted from the product of the individual plant yield × the number of plants per hectare and adjusting the weight based on 15.5% moisture.

Nano iron: Nano iron of 30 nm size were used from Khazra cheleated nano Fertilizers Company.

Physiological experiment of iron toxicity:

Bioaccumulation of nano iron in maize seeds:

Random samples were collected from mature maize seeds and the residual nano iron were measured using Atomic Absorption Spectrometer. (Aguilar, 2013)

Laboratory of animals' group: The study was conducted in Iraqi Center for Cancer

Research and Medical Genetics, collected 15 white male mice for every group aged 6-8 weeks with equal weight (25-30 g) and were randomly divided into 3 groups:

First group: Control group where 5 mice were given distilled water.

Second croup: Nano iron was given for 7 days for 5 mice. The dose was dosed with iron nanoparticles at a concentration of (13.29, 8.5 and 11.56) mg L^{-1} for 7 days.

Third croup: Nano iron treatment group was given for 14 days for 5 mice. The dose was dosed with nano iron at a concentration of (13.29, 8.5 and 11.56) mg L^{-1} for 14 days.

Blood was collected 7 days after the last dose and serum was separated and liver functions aspartate aminotransferase AST, alanine aminotransferase ALT and alkaline phosphatase ALP and kidney functions Urea and Creatinine levels of enzymes **Superoxide dismutase** SOD and Catalase CAT enzyme activity and Malondialdehyde (MDA) and Glutathione reductase GR and **Glutathione** GSH were measured for all studied groups.

The mice were anesthetized before blood collection and about 1.5 ml of venous blood was obtained for each mouse by Cardiac puncture method. The serum was separated immediately after all samples.

The statistical program GenStat was used to study the effect of different treatments, as the laboratory experiment was in CRD with four repetitions, and the field experiment was in RCBD with three repetitions, with the effect of nano iron spray treatments, as for the physiological experiment for laboratory animals, the effect of Nano-iron concentrations and sampling dates on the studied traits was studied, which were distributed in RCBD, and the averages were compared using the LSD test (Kadem & Abed, 2018).

Results & Discussion

Results of the laboratory experiment:

It appears from the results of Table 2 that, the concentration of Nano-iron 150 mg L^{-1} gave the highest activity of maize seeds, as it gave the highest average of the standard germination percentage (95.96%), germination strength (2696) and field emergence percentage (92.50%) with a significant difference from the other concentrations including comparison treatment, which gave the lowest average by 85.17%, 1508 and 79.25%, respectively.

Table (2): Characteristics of the crop, its components, and the content of Iron nanoparticles in seeds

Nano-Fe (mg L^{-1})	Seed Viability			Yield and Components		
	Standard Germination (%)	Seedling Vigor	Field Emergence (%)	No. of grain (grain $plant^{-1}$)	500-Seed Weight (g)	Grain Yield (t ha^{-1})
0	85.17±12.11 c	1508±143 c	79.25± 22.65 c	408.7± 63.11 D	93.23±13.07 c	4.061±0.41 d
50	91.36± 9.08 b	2122±198 b	84.67±12.31 b	538.9±87.26 C	98.80±7.33 b	5.711±0.78 c
100	90.57±18.95 b	2207±208 b	86.36±8.44 b	573.7±78.02 B	103.09±23.62 a	6.309±0.09 b
150	95.96±21.36 a	2696±218 a	92.50±17.08 a	628.3±101.58 A	101.02±18.77 a	6.765±1.13 a
LSD 0.05	**4.35**	**86**	**5.37**	**24.0**	**3.95**	**0.325**

This is due to the role played by nano-iron in the seed germination mechanism, as we can conclude that nano-iron particles helped the seeds absorb water, increase and stimulate of some necessary enzymes for germination, increase the activity of seedlings, reduce free radicals, and thus increase the germination rate and field emergence (Al-Dawoodi &Al-Fahad, 2021; Al-Issawi & Al-Fahad, 2023 a,b; Altayy & Cheyed, 2023).

Results of the field experiment:

The results showed that increasing the concentrations of Nano-iron to 150 mg L^{-1} gave the highest average number of grains per plant by 628.3 grain $plant^{-1}$ with a significant difference from other of concentrations including comparison treatment, which gave the lowest average by 408.7 grain $plant^{-1}$. This superiority is attributed to the effect of iron on stimulating dormant flowers and helped in fertilization, and led to the distribution of nutritional quantities for all grains equally, and extending the period of accumulation of dry matter in the seeds (Janmohammadi *et al.,* 2016). Increasing the concentrations of nano-iron led to a gradual increase the weight of 500 g, the control treatment gave 93.23 g as compared to concentrations of 100 and 150 mg L^{-1} which gave 103.09 and 101.02 g, respectively. This superiority may be attributed to the high efficiency of Nano-iron in targeting functional work centers and the ease of penetrating cell membranes due to its Nano-size, which works to enhance physiological processes and cell division, which improves the growth characteristics of the crop and increases the amount of manufactured dry matter and its transfer to the grains (drainers), thus increasing the weight of the grains (Al-Shaheen *et al.,* 2020; Al-Fahad *et al.,* 2020). The results showed that increasing the concentrations of nano-iron gave a significant increase in the total grain yield, as compared to the control treatment which gave 4.061 t $ha^{-}$the highest concentration of iron of (150 mg L^{-1}) gave highest average of 6.765 t ha^{-1}. This superiority is attributed to the effect of iron in increasing the accumulation of photosynthetic products, which was positively reflected on the components of the yield (number of grains in the ear and weight of 500 grains), (Kobayashi *et al.*, 2019) which is reflected in increasing the total grain yield (Elsahookie & Cheyed, 2023), which is consistent with what was reached by (AL-Shumary *et al.*, 2019) and (Al-Shaheen *et al.,* 2020).

Bioaccumulation of Nano-iron in maize seeds

The results of the study showed that the control group was free of nano-iron, while the other the fouler application of nano-iron lead to presence of residues of nano-iron particles in grain, (Table 3), and Figur (1).

Fig. (1): Standard curve of iron nanoparticle concentration

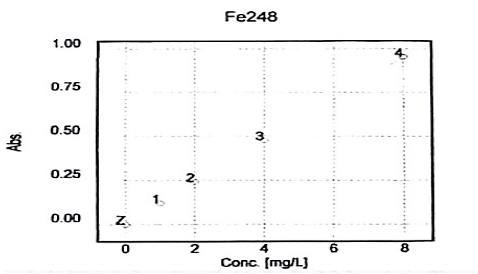

Table (3): Concentrations of nano-iron residues on maize seeds

Nano-iron treatment concentration (mg L^{-1})	Residual nano-iron concentrations in seeds) mg L^{-1})
control	0
50	13.29
100	8.5
150	11.56

Results of Nano iron toxicity

Effect of concentrations of nano-iron residues on liver and kidney functions:

The results of Table (4) showed that liver and kidney functions were affected by exposure to Nano-iron residues, and the toxicity was directly proportional to the increase in the duration of exposure. As for the concentrations, they showed a variation in the toxic effect according to the concentration. The concentration of (11.56) mg L^{-1} was the most toxic among all the concentrations studied, and its toxicity was directly proportional to the length of exposure to Nano iron, while the high concentration of 13.29 mg L^{-1} showed a significant toxic effect compared to the control treatment, but it had the least toxic effect compared to the other concentrations. While the lowest concentration used (8.5) mg L^{-1} showed a significant toxic effect compared to the control group and the highest concentration used (13.29) mg L^{-1}. The reason can be attributed to the possibility of molecules in low concentrations penetrating cell membranes, in addition to the possibility of molecules accumulating in high concentrations and transforming into molecular size and making it difficult for them to cross cellular membranes (Zanella *et al.,* 2017). This toxic effect is attributed to exposure to iron nanoparticles used in fertilizer (Waris *et al.,* 2023). The cytotoxic effect of the iron nanoparticles on liver enzymes is consequent to the harm effect of these molecules to the liver cells containing these enzymes. Any harm or injury to the liver cells will leading to the liberation of great amounts of liver enzymes into the streem blood (Li *et al*., 2023). Therefore, this harm to the cells can result from the raise level of their iron nanoparticles in the Kupffer cells of the liver that requirement a prolonged time to be ambidextrous to eliminate them up to 6 months (Uzhytchak *et al*., 2023).

Table (4) Effect of residual concentrations of iron nanoparticles in maize seeds on liver and kidney functions

Sampling Day	Nano-iron (mg L^{-1})	Liver and Kidney Functions (Mean ± SD)				
		AST (IU/L)	**ALT (IU/L)**	**ALP (IU/L)**	**Urea (mg/dl)**	**Crea (mg/dl)**
Control	**0**	33.00±7.35 C	45.33±10.51 D	13.30±1.58 C	32.30±10.61 D	0.500±8.61 B
7	**13.29**	42.67±15.39 B	63.00±13.77 C	29.70±3.84 B	58.00±13.30 C	0.670±0.15 B
	8.5	47.67±19.39 A	71.33±3.25 B	39.00±14.25 B	76.70±11.30 B	1.267±0.89 A
	11.56	50.33±21.99 A	85.00±0.14 A	61.30±24.37 A	85.30±18.14 A	1.533±0.77 A
LSD 0.05		3.76	3.33	18.14	12.23	0.605
14	**13.29**	71.00±18.36 b	99.00±31.22 C	89.67±27.84 b	90.30±27.64 b	1.90±0.21 C
	8.5	78.70±21.08 ab	118.00±43.33 B	93.00±30.03 a	99.00±32.39 ab	2.23±0.37 B
	11.56	83.30±23.64 a	129.00±39.11 A	99.33±21.44 a	116.00±42.17 a	3.90±0.49 A
LSD 0.05		9.97	18.66	6.62	13.13	0.78

Since the sinusoidal lumen of the liver makes them bared directly to the Nano-iron step inside the body and arrive the liver through the hepatic portal vein (Aboulhoda *et al*., 2023). When exciting the Kupffer cells, they manufacture a massive range of moderator like reactive oxygen species (ROS), nitric oxide, carbon monoxide and tumour necrosis factor TNF-α in increment to other cytokines. Consequently, it rules the inflammatory response to the liver. Any harme that affects the Kupffer cells will command to liver damage (Mirzajani *et al*., 2024). In addition, the efficiency of nanoparticles to prohibit the activity of antioxidant enzymes, mostly the superoxide dismutase (SOD) and glutathione transferase (GST) (Sokovnin *et al*., 2024). Consequently, the toxicity of these particles will be packing causing tissue deterioration and injure of the hepatic cells (Shabbir *et al*., 2023). Thence activating apoptosis (Hassan *et al*., 2020) due hepatic enzymes to be released into the blood.

Effect of residual concentrations of nano iron on oxidation enzymes:

The results of Table (5) showed a significant decrease in the level of antioxidants GR, GSH, SOD and CAT for all treatments with soaking seeds by Nano iron as compared to the control treatment. High concentrations of iron nanoparticles recorded a significant decrease in GR, GSH, MDA, SOD and CAT as compared to the control treatment. While MDA showed a significant increase for all soaking treatments as compared to the control.

Table (5): Effect of residual concentrations of Nano iron in maize seeds on oxidation enzymes

Sampling Time (Day)	Nano iron) mg L^{-1})	Oxidative Enzymes (Mean ± SD)				
		GR (Mmol/ml)	GSH (Mmol/ml)	MDA (nmol / mL)	SOD (nmol/L)	CAT (U/L)
Control	**0**	73.67±16.56 A	79.67±15.08 A	0.767±0.11 A	3.60±0.74 CB	50.40±20.01 A
7	**13.29**	71.33±17.02 A	80.67±23.11 A	0.847±0.16 A	4.83±1.28 B	50.37±18.94 A
	8.5	66.33±14.27 B	81.67±20.38 A	0.763±0.13 A	4.30±0.95 BC	46.33±19.64 B
	11.56	66.00±15.84 B	85.00±25.36 A	0.943±0.22 A	7.40±1.77 A	42.33±15.98 C
LSD 0.05		**3.81**	**N. S**	**N. S**	**1.21**	**3.86**
14	**13.29**	60.67±11.79 a	99.00±21.36 A	0.910±0.23 a	1.97±0.21 a	41.00±17.06 A
	8.5	58.67±12.86 a	97.00±31.65 A	0.907±0.23 a	2.37±0.37 a	38.67±12.64 A
	11.56	52.67±12.56 c	98.33±30.79 A	1.067±0.33 a	2.20±0.19 a	35.00±11.79 B
LSD 0.05		**3.60**	**N. S**	**N. S**	**N. S**	**2.33**

The increase was directly proportional to the increase in the studied concentrations and the. The reason for this difference may be attributed to the interference of iron nanoparticles in oxidative pathways and oxidation-reduction reactions. (Lee *et al.,* 2023). Nano Iron have the potential to induce cytotoxicity, DNA damage, cell cycle arrest, and apoptosis in cells mediated through oxidative stress and ROS production. Thus, lead to induce defect in oxidative balance (Siddiqui *et al*., 2023).

Conclusion

This study showed the positive effect of nano-iron germination and seedling emergence of maize. Nano-iron led to increasing maize productivity and improving the studied characteristics. In other hand, the nano-iron had a toxic effect on the functions of the liver, kidneys and the oxidative balance of the body at animal's levels.

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Contributions of authors

Z. A. H, carried out the laboratory animal experiments, collected the data and constructed the idea and hypothesis for research.

A. CH. F, carried out the field experiment and collected the data.

S. H. C, planned the methodology; project administration, data analysis and wrote the manuscript.

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Conflicts of interest

The authors declare that they have no conflict of interests.

Ethical approval

All applicable national and international guidelines for the care and use of animals were followed.

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تأثير نقع بذور الذرة بسماد الحديد النانوي على الانبات والإنتاجية وخصائصها السمية على فسيولوجيا الحيوانات

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المستخلص: أجريت تجربة حقلية خلال الموسم الربيعي لعام 2021 في الحقل التجريبي لكلية علوم الهندسة الزراعية – جامعة بغداد بهدف معرفة تأثير نقع البذور بالحديد النانوي بتراكيز (0 و50 و100 و150 ملغم لتر$^{-1}$) في حيوية البذور وبزوغها الحقلي وحاصل الحبوب ومكوناته للذرة الصفراء ومحتوى الحبوب من الحديد النانوي، وقد أظهرت النتائج زيادة واضحة ومعنوية في مؤشرات الإنبات والبزوغ الحقلي وحاصل الحبوب ومكوناته مصحوبة بزيادة تراكيز الحديد النانوي إلى أعلى مستوياتها (150 ملغم لتر$^{-1}$)، علاوة على ذلك ظهور بقايا الحديد النانوي في حبوب النبات الجديد وكان لها تأثير سام على وظائف الكبد والكلى والتوازن التأكسدي للفئران المعملية.

الكلمات المفتاحية: مضادات الأكسدة، وظائف الكبد، العناصر الدقيقة، الجسيمات النانوية، تحفيز البذور.