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Study the Effect of Spraying Hatching Eggs with Nano Plant Extracts on Embryonic Growth Rate, Hatching Percentage and Hatched Chick Weights

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Abstract | This study was conducted in the animal field of the University of Karbala/ College of Agriculture from 12/20/2022 to 11/1/2023 to know the effect of spraying hatching eggs with nano extract of Moringa seeds and nano extract of pomegranate peels on hatching characteristics and chick weight, where the eggs were weighed and weighed 56.45 ± 3 grams, and then the eggs were randomly distributed into four treatments, with 60 eggs per treatment and three replicates, and the experimental treatments were divided into T1: Control treatment. T2: Spraying with distilled water. T3: Spraying with nano extract of Moringa at a concentration of 5 ml/ 100 ml/ water. T4: Treatment of spraying nano extract of pomegranate peels 5 ml/ 100 ml/ water. Then the eggs were transferred after spraying treatment, the eggs were transferred to the incubator and after the hatching process. The results indicated a significant improvement (P ≥ 0.05) in the hatching percentage with a significant decrease (P ≥ 0.05) in the number of embryo deaths in fertilized eggs T2, T3 and T4, A significant decrease (P ≥ 0.05) in the percentage of live chicks and incomplete hatching in T2 (T3 and T4) during the total incubation period for all treatments compared to treatment T1. It was also noted that all spray treatments were significantly higher (P ≥ 0.05) in the relative weight of hatched chicks with a highly significant improvement (P ≥ 0.01) in all treatments in the phenotypic characteristics of hatched chicks when compared to treatment T1.

Keywords | Nanoparticles, Embryonic, Growth, Chick, Weigh, Plant extract

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INTRODUCTION

Poultry is of economic importance and an essential source of many important nutrients such as proteins and minerals. The poultry industry is often exposed to danger as a result of infectious agents (pathogens, whether viral, bacterial or parasitic), causing huge losses (Ajafar *et al*. 2024; Khallil *et al*., 2022). To address this problem, antibiotics are used to avoid harmful and pathogenic effects, but sometimes there are side effects of antibiotics. Currently, herbal extracts, green nanoparticles, exogenous enzymes, and, amino acids are used globally instead of antibiotics by adding them to natural feeds to improve poultry production (Arif *et al*., 2022). Additions are now being used to poultry feed as aromatic herbs, herbal of oils and nanomaterials, and they are verified and useful as

nutritional supplements that are inexpensive and safe and provide good financial returns (Abd El-Hack *et al*., 2022).

In recent years, there has been remarkable progress on nanotechnology, especially the green method, and there has been a focus on nanoparticles whose size is less than 100 nanometers because of their ideal qualities in medical operations and drug delivery (Keat *et al*., 2015). The process of producing green nanoparticles is the best method used in obtaining nanoscale plant extracts due to its low cost and free of side effects (Makarov *et al*., 2014). The green synthesis process for the production of nanoparticles has for contemporary diseases, and it is characterized by unique characteristics that distinguish it from other methods (chemical and physical) being environmentally friendly and low cost (Padalia and Chanda, 2017).

In recent years, there has been significant progress in nanotechnology, especially the green method, and there has been a focus on nanoparticles that are less than 100 nanometers in size because they possess ideal qualities in medical operations and drug delivery (Keat *et al*., 2015).

The process of producing green nanoparticles is the best method used to obtain nano-plant extracts due to its low cost and free of side effects (Makarov *et al*., 2014). The green synthesis process for producing nanoparticles has an important role contemporary disease, and it is characterized by unique properties that distinguish it from other (chemical methods and physical) because it is environmentally friendly and low in cost (Padalia and Chanda, 2017).

The pomegranate is a fruit known for its health benefits, including important biological compounds (antioxidants) such as phenolic compounds and gallic acids to addition in bone collagen and acids found in pomegranate, punicalin, and other phenolic compounds (Singh *et al*., 2018). Recently, research has focused on using plant waste to find antioxidant compounds in order to recycle them and make them cheap. Hence, pomegranate peels were used in nanosynthesis because they contain antioxidant compounds. The use of plant extracts has a positive effect on chicken growth rate, feed intake, and feed conversion ratio. This is due to the active compounds in the extract, such as hydrolyzable phenolic acids, tannins, and flavonoids. These compounds have anti-inflammatory and antioxidant properties, thus improving gastrointestinal function, which is reflected in the absorption and digestion of nutrients. They also have a positive effect on the growth of beneficial bacteria in the small intestine of broiler chickens, which positively impacts nutrient absorption and overall health (Baqer *et al*., 2023).
Moringa is considered a substance with nutritional value, and interest in it has recently been very great, especially in India, Pakistan, the Philippines, the tropical regions, and Africa, due to its advantages, especially in the parts of the leaves, flowers, and the vegetative part (Anwar *et al*., 2005).

Many studies have shown that moringa leaves are an important source of carotene, protein, calcium, and potassium. Therefore, they are considered a rich source of antioxidants. Adding them to food products protects them from spoilage because they contain fats and types of antioxidants, such as ascorbic acid, flavonoids, and carotenoids (Siddhuraju and Beacker, 2003). Moringa is considered an important source of vitamins and amino acids, (Onunkwo and George, 2015) and moringa seed extracts contain unsaturated and saturated fatty acids, (Ogbunugafor *et al*., 2011) so they show effectiveness against free radicals and prevent the formation of stray oxygen species (Sofidiya *et al*., 2006).

In this experiment, spraying nano-extracts on hatching eggs was studied and its effect on the embryonic mortality rate, hatching rate and chick weight at one day of the experiment was determined.

MATERIALS AND METHODS

This study was conducted in the animal production hatchery affiliated to the University of Karbala/ College of Agriculture for the period from 12/20/2022 to 11/1/2023, where hatching eggs were obtained from the fields of laying hens in the area immediately after collecting them and placing them in the refrigerator until the second day, and the herd was fed on a productive feed containing 16.88% protein and a capacity of 2726 kg/ kg feed.

Methods of preparing moringa leaf extract and nano pomegranate peel extract

Pomegranate peels were obtained from the holy Karbala Governorate and dried for seven days at room temperature with daily stirring. After that, the peels were ground using an electric grinder to turn them into powder. Moringa leaves were obtained from the gardens of the College of Agriculture, University of Karbala, and were dried and ground using a small electric grinder to form a powder.

Also, dried Moringa powder was used using a grinder to obtain powder. 20 grams of powder were taken, and 400 ml of distilled water was added to it. After that, the solution was placed on a magnetic shaker at a temperature of 100/ dm for 20/min. Then, it was filtered using filter paper No. 1. We take 10 ml of the filtrate and add 90 ml of silver nitrate solution to it Then we weigh 1.6987. We dissolve it in a liter of distilled water on a magnetic shaker, and the addition is done gradually at a temperature of 30 degrees

Celsius for 30 minutes. The color of the solution changes to brown. We put the mixture in the centrifuge for 20 minutes and 1200 rpm.

AgNO3 (0.1 mmol) was mixed with 250 ml of DDH2O, then ten ml of pomegranate peel extract was added, and the solution was mixed well using a shaking incubator for 5 min.

The results of the reaction mixture were its color changed from a colorless solution to a brown solution after 24 hours, indicating the reduction of silver ions into silver nanoparticles. The nanoparticle solution was centrifuged for 15 minutes at a speed of 15,000, and the process was repeated 4 times. After that, the AgNPs were collected, and then assays were conducted to analyze the properties and biological activities of the synthesized AgNPs.

How to spray hatching eggs

In the current study, 500 eggs with an average of 56.45 grams were used, collected from the field of laying hens and distributed into 4 groups. 3 balls per group subgroups: one group was sprayed with distilled water, another with nano-pomegranate peel extract, a group with nano-moringa leaf extract, and an untreated "control" group.

Hatching process

The eggshells were sterilized with 100% ethanol, and then the eggs were distributed on the trays. Then the egg surfaces were sprayed with distilled water, pomegranate peel extract, anise extract, clove extract and untreated control group. The incubator conditions were 37.5°C, 55-60% relative humidity, and 37.0°C. The egg groups were transferred to the incubator. Each replicate consisting of 60 eggs (three per replicate) were placed in the egg trays. After cleaning the egg surface with 100% ethanol, the egg hell was sprayed with distilled water, anise extract, pomegranate peel extract, clove extract and untreated control groups. The temperature and relative humidity were maintained at 37.5°C and 55-60%, respectively.

Characterization of nanoparticles

Scanning electron microscopy (SEM) (pomagranate nanoparticles)

By measuring the scanning electron microscope, it is possible to determine the shape and size of nanoparticles and their distribution with high accuracy and magnification power. Scanning electron microscope images of nanoparticles show the distribution of atoms that are in the form of spherical grains with an average grain size (18.49 nm–21.08 nm) (pomagranate nanoparticles) (Figure 1A, B) (13.33nm-14.79nm) moringa nanoparticles SEM (Figure 1A, B), and these grains are homogeneous and non-contiguous.

TEM characterization of the synthesized NPs

Nanoparticles' composition and size distribution might be seen using a transmission electron microscope. The TEM and particle size distribution images of NPs obtained in this investigation are presented in (Figure 1A, B), with particle sizes ranging from 15.85-19.81 nm.

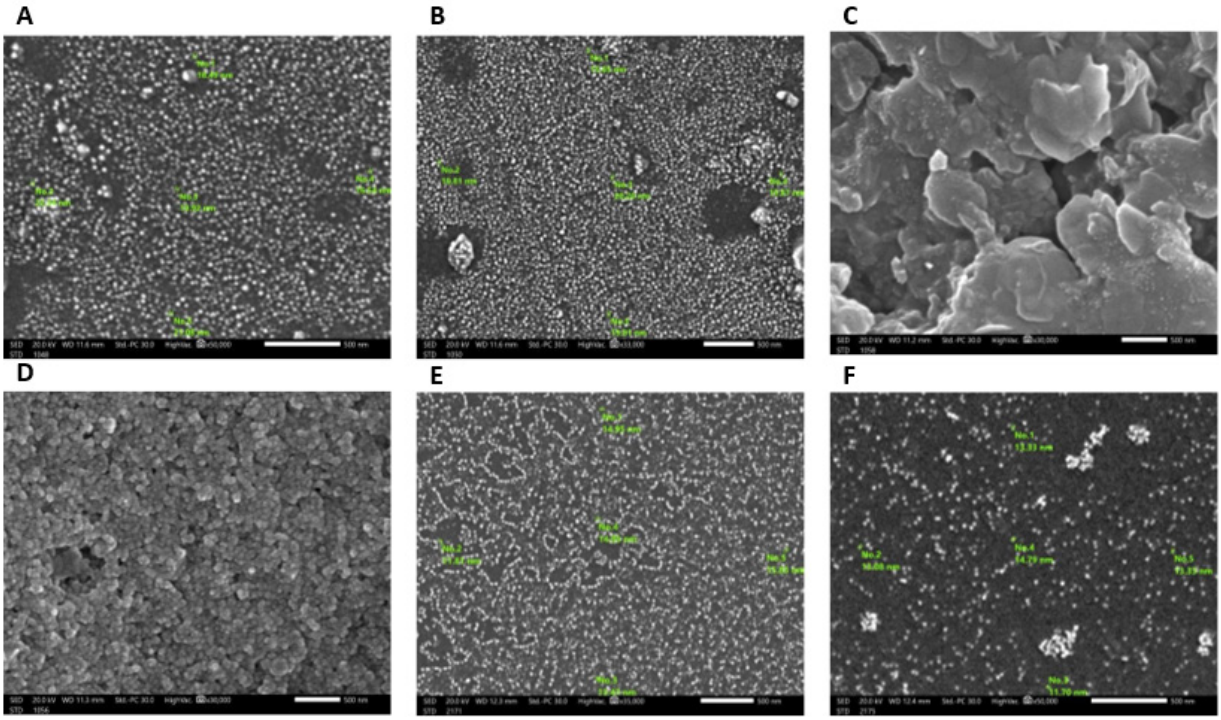

Figure 1: SEM and TEM images of green-synthesized nanoparticles from pomegranate peel (A, B), Moringa leaves (C, D), and their TEM characterization (E, F), showing uniform spherical morphology and particle sizes ranging from 13.33 to 21.08 nm.

RESULTS AND DISCUSSION

With regard to the hatching rate, we note from Table 2 and Table 3 that the highest significant superiority (0.01 ≥P) in the hatching rate was obtained for treatment T4 compared to treatments T1, T2, and T3 on the basis of fertilized eggs, while we find significant differences between treatments T1, T2, and T3, while an arithmetic superiority was found. For spraying treatments with aqueous plant extracts, T3 and T4, compared to the control treatments T1 and T2.

Several studies have reported the antibacterial effect of Morinca seeds and their high content of antioxidants. These are the properties of Morinca seeds, as well as the fact that they contain lipophilic compounds that can effectively bind to the cell membrane (Jabeen *et al.*, 2008).

Luqman *et al.* (2012), stated that Morinca seed extracts contain antibiotics such as carboxylic acid and 2,4-diacetylphloroglucinol, and it was noted that there are enzymes that degrade the cell wall. The antioxidant effect of Moringa oleifera seeds is due to the presence of polyphenols, which have the ability to remove free radicals such as tannins, anthocyanins, glycosides and thiocarbamates.

The outer shell of the pomegranate fruit contains many alkaloids, including pelleterin. This substance was also found with other alkaloids, namely, N-ethyl methyl isopilliferin, pseudopillitecrine isopilliferine, ethyl pillitecrine, (Gatea *et al.*, 2019; 2023; Kadhim *et al.*, 2021) confirmed in many studies that the pomegranate fruit has a lethal and inhibitory effect on the growth of swimming microbes that feed on gram dye and a number of fungi.

Prashanth *et al.* (2001) that spraying broiler eggs with water or natural extracts can improve hatchability by reducing embryonic mortality during different incubation periods, thus enhancing the use of chicken eggs in hatcheries (Baqer al et., 2023; Ajafar *et al.*, 2024).

Table 1: The effect of spraying hatching eggs with some nano-extracts on the rate of embryonic mortality during the embryonic development period ± standard error.

Traits	Early embryonic mortality %	Intermediates embryonic mortality %	Late embryonic mortality %
T1 Control	(0-7)	(8-15)	(16-21)
T2 Spraying distilled water	3.2	0.0	0.0
T3 Spraying nano moringa extract	2.6	0.0	5.2
T4 Spraying nano pomegranate peel extract	0.0	0.0	2.5

Table 2: The effect of spraying broiler hatching eggs with some nano-extracts on the hatching percentage ± standard error.

Traits	Hatchability %
T1 Control	0.170±94.77 B
T2 Spraying distilled water	0.352±92.64 C
T3 Spraying nano moringa extract	0.050±91.50 C
T4 Spraying nano pomegranate peel extract	0.570±97.50 A
significantly difference	0.01
LSD	1.0074

Table 3: The effect of spraying hatching eggs with some nano-extracts on the weights of hatched chicks (g) at the age of one day ± standard error.

Traits	Chick weight at hatch (g)
T1 Control	38.26± 0.70
T2 Spraying distilled water	39.86± 0.72
T3 Spraying nano moringa extract	38.98 ± 1.25
T4 Spraying nano pomegranate peel extract	38.48 ± 1.57
Significantly difference	N.S
LSD	3.277

Nanoparticles based on pomegranate peel extract and moringa seeds were prepared. The formulations were silver nanoparticles based. Nanoparticles have shown promising antibacterial and antifungal activity. Also, these herbal nanoparticles are safe, economically cheap, and easy to use (Vinay *et al.*, 2018; Salman *et al.*, 2024a, b).

CONCLUSION

The results indicated a significant improvement (P ≥ 0.05) in hatchability with a significant decrease (P ≥ 0.05) in the number of embryonic deaths in T2, T3 and T4 fertilized eggs. It was also noted that all spray treatments had significantly higher (P ≥ 0.05) relative weight of hatched chicks with a highly significant improvement (P ≥ 0.01) in all treatments in the phenotypic characteristics of hatched chicks when compared to the control treatment T1.

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NOVELTY STATEMENT

This study is the first to investigate the impact of nano-extracts derived from Moringa seeds and pomegranate peels on hatching outcomes through direct egg spraying, offering an eco-friendly alternative to antibiotics in poultry hatcheries.

AUTHOR'S CONTRIBUTION

HAA: Data collection and laboratory analysis.
ABJ: Conceptualization, methodology, and manuscript preparation.
HQB: Statistical analysis and figure development.

Compliance with ethical standards

This article contains no studies involving human or animal subjects.

Generative AI and AI-assisted technology statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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