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Preparation of Fortified Edible Gelatin Films Nanoscale Magnesium Oxide and Study of It's Mechanical and Antibacterial Effectiveness

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Abstract: Membranes were prepared from laboratory bovine gelatin with polyvinyl alcohol in fixed proportions of 1/1 (wt/wt), using plasticizers (glycerol and sorbitol), adding magnesium oxide nanoparticles to them, and comparing them to unreinforced membranes, and measuring the mechanical properties of these membranes, which were the thickness of the membrane and tensile capacity. In addition to elongation, solubility, and antimicrobial activity. It was noted that the thickness of laboratory bovine gelatin membranes supported with magnesium oxide nanoparticles is 30 mm, which is more than the thickness of the unsupported gelatin membrane, which is 6 mm. The results indicated that the greatest tensile strength (γM Scma Max) that the model could withstand (the strength of the model) was for the membrane made from laboratory gelatin with 1 gm of polyvinyl alcohol (PVA), 18 gm of glycerol, and 10 ml of nano solution, and it reached 117 MPa with a pouring ratio of 10 ml. However, the lowest tensile strength (σM) was 0.0705 MPa for the membrane made from laboratory gelatin with 1 gm polyvinyl alcohol PVA, 18 gm glycerol, and 10 ml of nano solution with a casting ratio of 15 ml, and the best flexibility (the greatest elongation) (Epsilon At break (εM % Upsilon at break) was for the membrane made from laboratory gelatin with 1 g of polyvinyl alcohol (PVA), 18 g of glycerol, and 10 ml of nano solution, and it reached 117 MPa with a pouring ratio of 43.3 with 10 ml. It was noted that the solubility of the membranes supported with magnesium oxide nanoparticles was lower than the solubility of the membranes not supported with magnesium oxide nanoparticles. It was found that there was a high inhibitory activity for nanogelatin membrane solutions compared to membrane solutions not supported by nanoparticles against all types of selected pathogenic bacteria. The highest inhibitory activity obtained for the nanogelatin membrane was against *Staphylococcus aureus* bacteria, then against *Escherichia coli* and *Pseudomonas aeruginosa* bacteria, respectively, although less. The effect of gelatin film on *Escherichia coli* bacteria.

Key words: Edible reinforced films, nanopolymers, antimicrobials, mechanical properties.

Introduction: Choosing the ideal packaging materials represents a challenge to replace the non-biodegradable, petrochemical-based plastics used worldwide, which are primarily responsible for environmental pollution. Approximately 380 million tons of plastic materials are produced annually around the world, and approximately 40% are used. For food packaging, massive campaigns against the use of plastics have been stimulated all over the world, creating a need to adopt more sustainable and environmentally friendly food packaging alternatives, especially those made from biodegradable biopolymers. Gelatin is a soluble protein resulting from the thermal denaturation of collagen from polymers. Therefore, gelatin has been widely studied for its usefulness as a food packaging material due to its low cost, susceptibility to biodegradation, safety, and non-toxicity [1;2]. Edible packaging is known as a potential alternative to protect food quality and prolong shelf life by delaying microbial spoilage and providing good properties that prevent the exchange of moisture and gases with the external environment. [3]. Edible films are biopolymers that are manufactured to preserve and package food. They work to prevent the passage of gases, vapors, and fatty materials. They also prevent the loss of flavor materials and improve the external appearance of the product, making it more attractive to consumers [4]. These films transferred packaging to A higher level, as the concept of food, preservation and packaging is combined in a biodegradable and edible membrane. It also prevents discolouration, fat oxidation and unpleasant odors, enhances shelf life and can act as carriers of active agents such as antimicrobials, antioxidants, flavors and nutrients and contributes to the preservation of meat, fish and products derived from them. [5]. The application of nanotechnology in food packaging allows it to have a greater degree of protection by increasing its thermal and mechanical capabilities and antibacterial and antioxidant properties [6]. Given that fish is a highly perishable food item, and in order to reduce the economic losses of fish traders, the study aimed to use edible films containing magnesium oxide nanoparticles that have the ability to inhibit the growth of microorganisms and work to extend the preservation period from 6 to 13 days for the coated fish fillets, depending on the type of active ingredients integrated with the membrane [7].

Experimental Methodology

Preparation of gelatin membrane : The membrane solution was prepared according to the method [8],with some modifications, as gelatin was used with polyvinyl alcohol in fixed proportions of 1/1 (weight/weight) with the use of plasticizers (glycerol and sorbitol), and after conducting a series of experiments to choose the most suitable plasticizer, the glycerol plasticizer was adopted in preparing the membrane (it was conducting a series of experiments to choose the appropriate concentration (1 g) of polyvinyl alcohol was dissolved with distilled water, then mixing was carried out using a stirrer hot plate – Magnetic stirrer until complete dissolution at a temperature of 90°C. After complete dissolution, it was left to cool at room temperature. Gelatin was prepared by dissolving 1 g of it with distilled water with manual stirring first, then stirring it using a hot plate magnetic stirrer until complete dissolution. Then the gelatin and polyvinyl

alcohol were combined together with continuous mechanical stirring using a magnetic mixer, then glycerol (18 g) was added (done Conduct a series of experiments to choose the appropriate size (with continuous stirring without heat for 30 minutes, then pour the solution into plastic Petri dishes with a diameter of 9 cm and 10 and 15 ml for each dish (after performing several experiments to obtain the appropriate thickness of the membrane), and the surface level was adjusted using For balance, the dishes were left to dry at room temperature for 48 hours. After drying, the films were removed from the dishes and kept in polyethylene bags and kept in the refrigerator until use. Preparation of gelatin membrane supported with nanomagnesium oxide solution Preparation of gelatin membrane supported with nanomagnesium oxide solution The nanofilm was prepared by dissolving a volume of 0.001 g of nanomagnesium oxide with 10 ml of distilled water and adding it to a mixture (1 g of polyvinyl alcohol, 1 g of gelatin, and 18 g of glycerol) with continuous mechanical stirring without heat. Preparation was carried out as in the previous step. Mechanical checks of the membrane determination of thickness of film The thickness of the membrane was estimated using a manual micrometer with an accuracy of 0.01 mm (for nanoparticle-supported and non-supported membranes) and according to the method of [9], by choosing five random locations of the membrane from the periphery to the center, then the average readings were calculated to represent the thickness of the membrane. This examination was conducted in a research center. Polymer/University of Basrah.

Estimating tensile strength and percentage elongation at break

The tensile strength and elongation ratio of nanoparticle-supported and non-nanoparticle-supported films were estimated according to the method described by [10]. They used the Textur analyzer of the Polymer Research Center/University of Basra and based on the standard method of the American Society for testing and materials described by [11]. The films were cut in the form of rectangular strips (10 × 1 cm). (Several layers of adhesive tape were wrapped on both ends of the membrane tape to ensure that it was firmly attached between the two handles of the device. The withdrawal rate for the samples was 50 mm/min, the cutting speed was 200 mm/min, and the device speed was 5 mm/min. The tensile strength and elongation at cutting were estimated from the (stress and ductility curves.) Stres-strain curve which the device drew for the samples. Determination of film solubility The water solubility of the films was determined according to the method of [12] by immersing the membranes in distilled water for 24 hours, then calculating the percentage of dissolved membrane material. The membranes were cut into square pieces with dimensions of 5 x 5 cm, dried at a temperature of 105 °C, weighed accurately with a sensitive balance, and then immersed again in 50 ml of distilled water. At a temperature of 25°C, it was dried again at the same temperature and reweighed to obtain the final weight, and the following equation was used to estimate the percentage of solubility. % solubility = initial weight of sample - final weight of sample x 100 Initial weight of the sample Inhibitory activity of gelatinous membrane supported and unsupported with magnesium oxide nanoparticles The inhibitory effectiveness was estimated according to the method of [13]. All culture media were

prepared in the laboratory according to the instructions of the producing company and sterilized in an incubator at a temperature of 121°C and a pressure of 1.5 pounds per inch for a period of 15 minutes. Several types of bacteria were tested, which were obtained from the University of Basra/College of Science. /Department of Life Sciences, which are: *Esherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*.

Determination of thickness of film

The thickness of laboratory bovine gelatin membranes supported and unsupported with magnesium oxide nanoparticles was estimated as in Figures (1 and 2). It was observed that the thickness of the membrane increased in the presence of nanoparticles, as the thickness of the unsupported gelatin membrane was 6 mm while the thickness of the supported membrane was 30 mm.

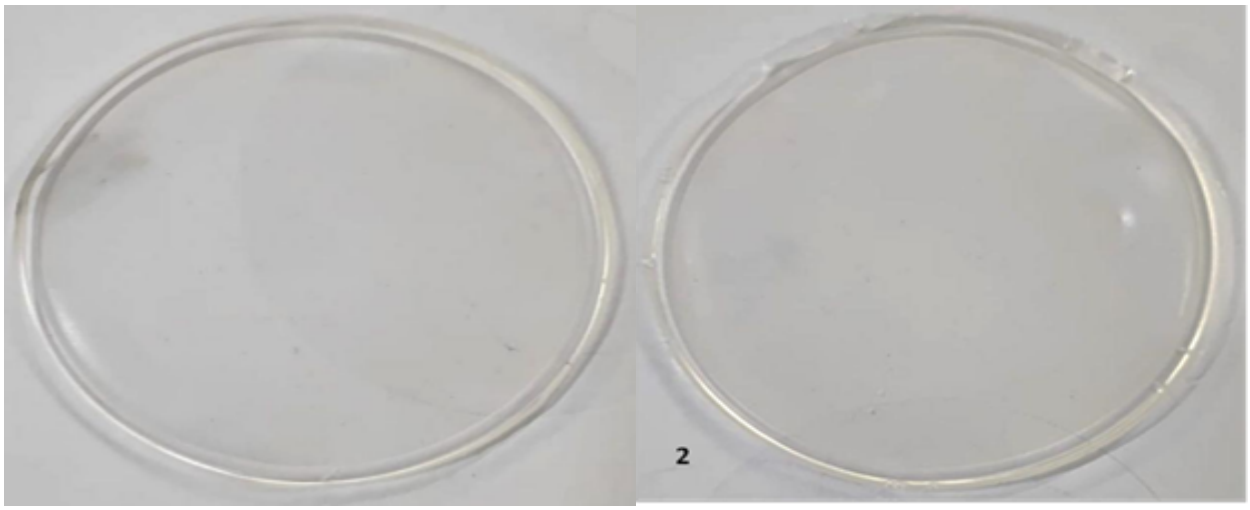

Figure (1 and 2): (1) -Gelatin membrane only(2) - Gelatin membrane supported with Nano-MgO.

Estimating tensile strength and percentage elongation at break

The tensile and elongation properties of the manufactured films were measured using a German-made Tensile strength and elongation measuring device. The measurement was done at the Polymer Research Center - University of Basra. 10 ml and 15 ml of membrane solution were taken for the examination. These membranes prepared from reinforced and unsupported laboratory gelatin were good from a theoretical and practical point of view. The results showed that the greatest tensile strength (γM Scma Max) that the model could withstand (the strength of the model) was for the membrane made from laboratory gelatin with 1 gm polyvinyl alcohol (PVA), 18 gm glycerol, and 10 ml of nano solution with a pouring ratio of 15 ml, and the best flexibility (the greatest elongation) (Epsilon et Break (γM % Upsilon at break) for the laboratory gelatin with 1 gm of polyvinyl alcohol (PVA), 18 gm of glycerol, and 10 ml of nano

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solution, and it reached 117 MPa with a pouring ratio of 10 ml. However, the lowest tensile strength (γM) was 0.0705 MPa for the membrane

membrane made from laboratory gelatin with 1 gm polyvinyl alcohol PVA, 18 gm glycerol, and 10 ml of nano solution reached 117 MPa with a pouring ratio of 10 ml and reached 43.3, Table (1) and Figure (3) and (4). This is due to the high plasticizing power of glycerol and the hydroscopic properties of glycerol due to its association with water molecules that have an additional plasticizing effect. The plasticizing power of glycerol was attributed to the small size of the glycerol molecules (18 g/mol), facilitating the penetration process between the polymer molecules and plasticizing events

Table (1): Tensile strength and elongation of manufactured membranes supported and not supported by nanoparticles.

Membrane Type	Pouring Ratio (Ml)	A_0 Mm^2	B Mm	H Mm	$\square_B$ %	$\square_B$ Mpa	$\square_M$ %	$\square_M$ Mpa	$\square_Y$ Mpa	E_t Mpa
A	10	0.70	14	0.05	0.9	3.29	0.8	3.90	-	29.1
	15	27.00	18	1.5	55.3	0.0545	43.4	0.0980	-	0.0336
	30	5.10	17	0.3	1.0	4.89	1.0	4.98	-	1180
B	10	0.70	14	0.05	1.7	117	1.7	117	-	871
	15	27.30	13	2.1	-	-	-0.0	0.0705	0.0226	-0.154
	30	6.00	20	0.3	1.8	15.0	1.8	15.4	-	660

a- A solution of the membrane made from laboratory gelatin with 1 g of polyvinyl alcohol (PVA) and 18 g of glycerol.

b- A solution of the laboratory gelatin membrane with 1 g of PVA, 18 g of glycerol, and 10 ml of the nano solution.

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Statistics table of Tensile strength:

Series	E_t	σ_Y	σ_M	ε_M	σ_B	ε_B	H	B	A_0
N = 4	Mpa	Mpa	Mpa	%	Mpa	%	Mm	Mm	Mm^2
X	225	-0.0226	30.3	11.5	40.1	19.3	0.925	14.75	13.93
S	431	-	57.9	21.3	66.7	31.2	1.04	2.217	15.27
□	-	-	191.14	-	166.03	161.33	112.39	15.03	109.67

Statistics table of Tensile of elongation:

Series	E t	σ_Y	σ_M	ε_M	σ_B	ε_B	h	B	A_0
n = 4	Mpa	MPa	MPa	%	Mpa	%	mm	mm	mm^2
x	225	-0.0226	30.3	11.5	40.1	19.3	0.925	14.75	13.93
s	431	-	57.9	21.3	66.7	31.2	1.04	2.217	15.27
□	-	-	191.14	-	166.03	161.33	112.39	15.03	109.67

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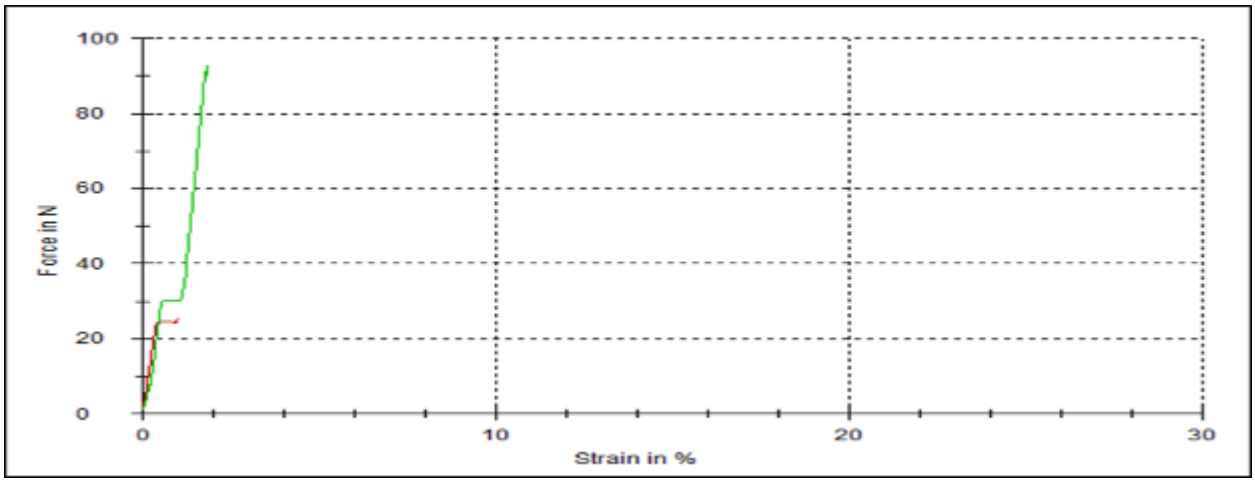

Figure (3): The membrane made from laboratory gelatin with 1 g of polyvinyl alcohol (PVA) and 18 g of glycerol.

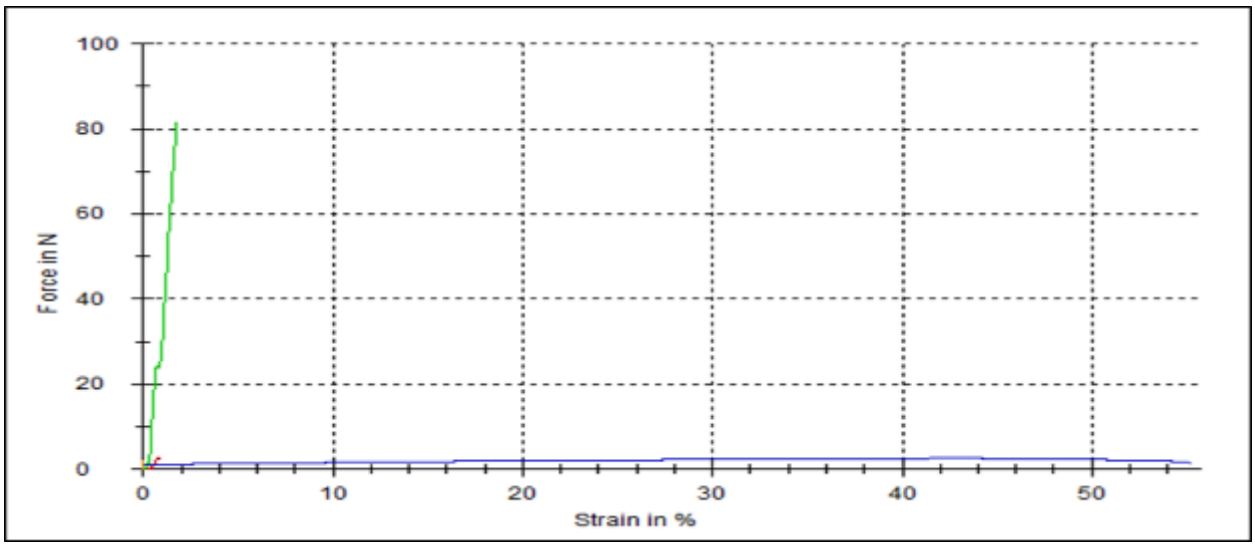

Figure (4): A membrane made from laboratory gelatin with 1 g of polyvinyl alcohol (PVA) and 18 g of glycerol supported by nanoparticles.

Determination of the solubility of films supported and unsupported with magnesium oxide nanoparticles.

Determination of film solubility

The results are shown in Table (2) of the solubility percentages of membranes supported and not supported with magnesium oxide nanoparticles, as it was noted that the solubility of membranes supported with magnesium oxide nanoparticles was less than the solubility of membranes not supported with magnesium oxide nanoparticles. The reason for the decrease in the solubility of

the reinforced films may be due to the interaction between the polymer chain and the nanoparticles, as the movement of the chains was restricted and thus the solubility of the coating was reduced [14].

Table (2): Ability of membranes supported and unsupported with magnesium oxide nanoparticles to dissolve in water

mL	Solubility %	
	A	B
1	92	90
2	90	89
3	91	90
4	89	88
5	92	90
6	91	91
7	91	90
8	91	90
9	90	90
10	91	90

a- A solution of the membrane made from laboratory gelatin with 1 g of polyvinyl alcohol (PVA) and 18 g of glycerol.

b- Laboratory gelatin membrane solution with 1 g PVA, 18 g glycerol, and 10 ml nano solution.

Inhibitory activity of the membrane against microorganisms:

The results in Figures (6), (7) and (8) showed a difference in the effect of the inhibitory action of gelatin membrane solutions supported and not supported with nanoparticles against the Gram-negative and positive pathogenic bacteria under study. It was noted that there was a high inhibitory activity for nanogelatin membrane solutions compared with solutions of The membranes not supported by nanoparticles against all types of selected pathogenic bacteria, and the highest inhibitory activity was obtained for the nano-gelatin membrane against Staphylococcus aureus bacteria, then against *Escherichia coli* and

Pseudomonas aeruginosa bacteria, respectively. The gelatin membrane had the least effect on Escherichia coli bacteria. The higher inhibitory effectiveness on *Staphylococcus aureus* bacteria compared to other types of bacteria may be due to the fact that its wall consists of a single layer [15]. The antimicrobial effectiveness of gelatin is due to its cationic property and NH3 groups that bind with negative ions on the surfaces of bacterial cells. It is believed that they bind with sialic acid and the bonds are cross-linked, which impedes the movement of nutrients into the cells, the penetration of gelatin molecules and their interference with the cell's nuclear material,

DNA, and inhibiting the synthesis of mRNA, as well as causing minerals to retain and bind with essential nutrients for microbial growth, thus preventing their transfer into the cell and thus its death [16] [17], and chitosan also affects bacterial membranes, causing their damage [18]. The high inhibitory effectiveness of nanomembrane solutions against pathogenic bacteria may be due to several hypotheses, the most important of which are damage to the cytoplasmic membrane, increased membrane permeability, a direct effect on metabolism, and inhibition of cell extrinsic enzymes [19].

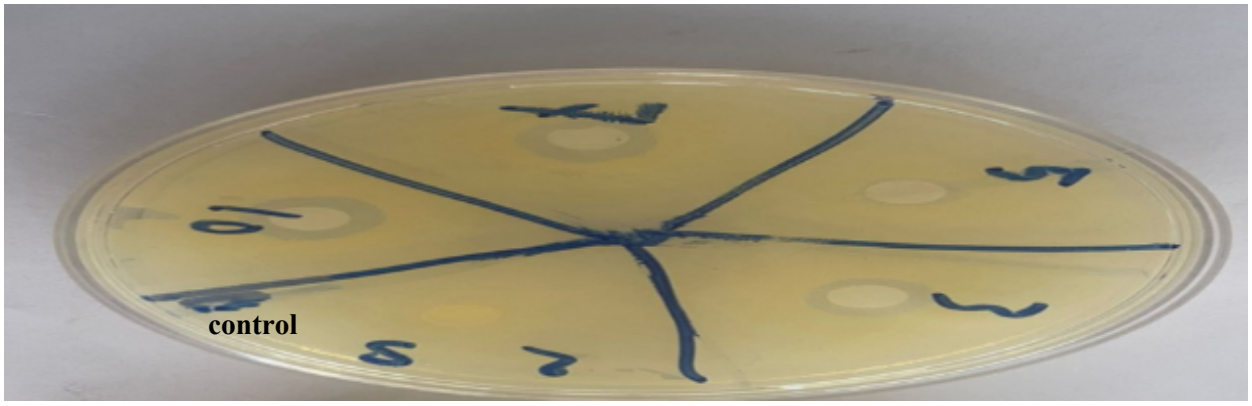

Figure (5): Inhibition test for bacterial growth and biological effectiveness against *Staphylococcus aureus* bacteria, the control is not supported with MgO nanoparticles.

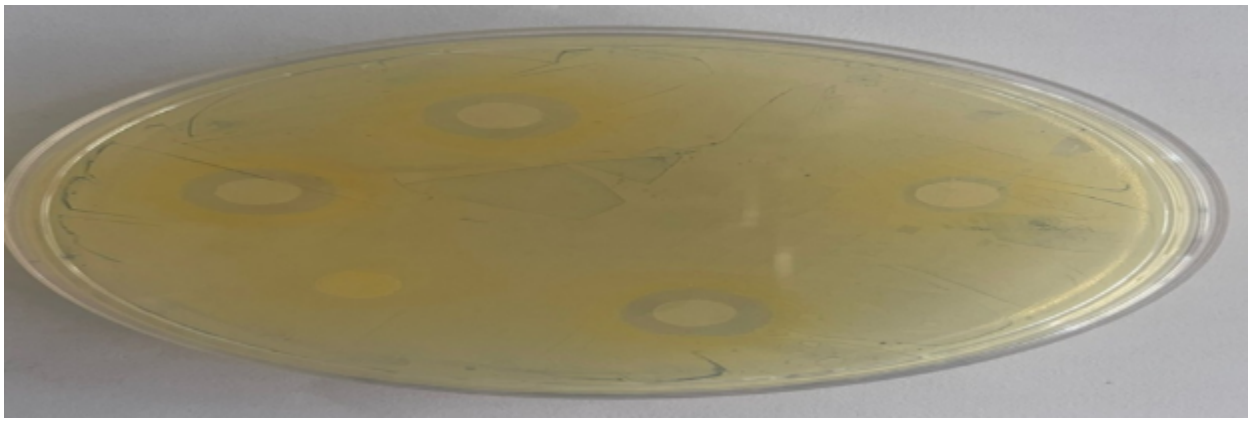

Figure (6): Inhibition test for bacterial growth and biological activity against *Escherichia coli* bacteria, the control is not supported with MgO nanoparticles.

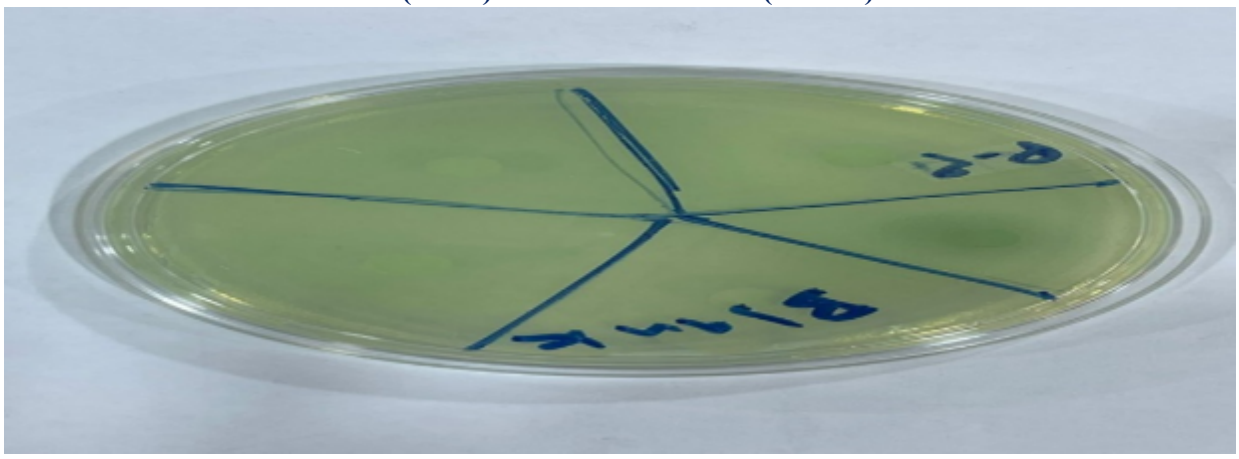

Figure (7): Inhibition test for bacterial growth and biological activity against *Pseudomonas aeruginosa* bacteria, the control is not supported with MgO nanoparticles.

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