

EFFECT OF NANO SILVER (AG NP-124) AS EGG SHELL SANITIZER ON THE KEEPING QUALITY OF TABLE EGGS

C. Pandian^{*1}, R. Richard Churchill², S. Manoharan³ and P.Veeramani⁴

Veterinary University Training and Research Centre
Tamil Nadu Veterinary and Animal Sciences University
Vellore – 632 009

ABSTRACT

Nano silver particles are valuable alternative for antibiotics and disinfectants as it is relatively free of adverse effects. Nano silver particles (Ag-NPs) have new physical and chemical characteristics enabling it to have a strong antibacterial activity and used today more as a high disinfectant in poultry farms. This study was aimed to evaluate the effect of different concentrations of 10, 20 and 50 ppm of Ag-NPs as egg shell sanitizer and assess the microbial count on the egg shell surface during different storage conditions. The total bacterial count on the egg shell and egg quality characteristics were carried out on 1, 7, 14, 21 and 28 days of storage after sanitation. Presence of nano silver and distribution on egg shell surface were determined using transmission electron microscopy. Results showed that bacterial loads on the egg shell were declined with the increased concentration of Ag-NPs used and the 50 ppm concentration of Ag-NPs had 1-3 log reduction in the total bacterial count on one day after disinfection in the egg shell surface which were comparable with the positive control (1% Kohrsolin) used in the trial. However, the external and internal qualities of eggs did not show any significance difference among different treatments during different storage days. However, nano silver 50 ppm had comparatively better zone of inhibition than 10 and 20 ppm levels in disc diffusion method. This study concluded that using Ag-NPs at 50 ppm concentration in disinfecting chicken egg can effectively reduce bacterial load on egg shell.

Key words: Nano silver, egg shell sanitizer and keeping quality

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¹Associate Professor and Head, Corresponding author Email id: chinnaduraipandian75@gmail.com

²Professor and Head, Department of Poultry Science, Madras Veterinary College, Chennai – 600 007

³Professor, Vaccine Research Centre – Bacterial Vaccine

⁴Professor and Head, Livestock Farm Complex, Veterinary College and Research Institute, Salem - 636 112

INTRODUCTION

Microbial contamination is considered as a serious issue in food industries. In recent years, disease outbreaks associated with bacterial contamination of egg and egg products were reported worldwide (Sabarinath *et al.*, 2009). Several studies have reported a high incidence of bacterial contamination in eggs stored in retail outlets on reusable egg trays without proper hygiene. It is also suggested that reduction of bacterial contamination will help to increase the shelf-life of the eggs (Bertechini and Mazzuco, 2013).

Nano silver has got antimicrobial properties, i.e. anti-bacterial antifungal and antiviral with no harmful side effects (Wright *et al.*, 1999 and Bhanja *et al.*, 2019). The silver ions released from the surface of nano silver control and destroy microorganisms, due to their binding to the bacterial cells membrane (Fernandez *et al.*, 2010). Under this circumstance, nanoparticles are considered as an alternative for traditionally used disinfectant, as well as potentially used on a wider scale in poultry industry including hatcheries. The aim of this proposed research is to evaluate the effect of nano silver (AG-NP-124) as egg shell sanitizer and assess the microbial count on the shell surface during different storage conditions.

MATERIALS AND METHODS

Preparation of nano silver in the stock solution

The experiment was conducted at the department of Poultry Science, Madras Veterinary College, Tamil Nadu Veterinary

and Animal Science University, Chennai- 600 007 during the year 2021. The concentration of nano silver in the stock solution was estimated to be 160.73 ppm using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) method. The working concentration of 10 ppm (31.10 ml of nano silver stock solution + 468.89 ml of triple distilled water), 20 ppm (62.21 ml of nano silver stock solution + 437.78 ml of triple distilled water) and 50 ppm (155.54 ml of nano silver stock solution + 344.45 ml of triple distilled water) of nano silver were used to assess the effectiveness as egg shell sanitizer.

Experimental design

A total of 300 freshly laid chicken eggs with uniform weight were purchased from a commercial layer farm. The eggs were then randomly distributed among five treatment groups each with 6 replicates. Group 1 formed the negative control as the untreated group (T_1). Group 2 formed the positive control, where the eggs were treated with commercial egg shell sanitizer namely Kohrsolin TH[®] (T_2). (It contains glutaraldehyde, 1, 6- dihydroxy 2, 5- dioxahexane and polymethylolurea derivatives). Groups 3 to 5 were treated with nano silver (AG-NP-124) coating in three different concentrations such as 10 (T_3), 20 (T_4) and 50 ppm (T_5) over the eggs. One set of 150 eggs stored at room temperature (25°C) and another set of 150 eggs stored at refrigeration temperature (4° C) for 28 days. The total bacterial count on the egg shell and egg quality characteristics were carried out on 1, 7, 14, 21 and 28 days of storage after sanitation (Table 1).

Table 1. Experimental design to assess microbial count on egg shell and egg quality characteristics in different storage condition after sanitation

Storage days	Storage condition									
	Room temperature (N=150 eggs)					Refrigeration temperature (N=150 eggs)				
	T ₁ -ve control	T ₂ +ve control (Kohrsolin TH 1 %)	T ₃ 10 ppm (Nano silver)	T ₄ 20 ppm (Nano silver)	T ₅ 50 ppm (Nano silver)	T ₁ -ve control	T ₂ +ve control (Kohrsolin TH 1 %)	T ₃ 10 ppm (Nano silver)	T ₄ 20 ppm (Nano silver)	T ₅ 50 ppm (Nano silver)
1	6	6	6	6	6	6	6	6	6	6
7	6	6	6	6	6	6	6	6	6	6
14	6	6	6	6	6	6	6	6	6	6
21	6	6	6	6	6	6	6	6	6	6
28	6	6	6	6	6	6	6	6	6	6
Total eggs	30	30	30	30	30	30	30	30	30	30

Examination of nano silver doped on egg shell surface viewed by Transmission Electron Microscope

Presence of nano silver and distribution on egg shell surface were determined using transmission electron microscopy.

Microbial count on the egg shell surface

The total bacterial count on the egg shell was studied to assess the keeping quality of table eggs using the test compound Ag NP-124 as egg shell sanitizer. Egg wash method has carried out to harvesting the bacteria present on the egg shell surface. In this method, egg was placed in a zip lock cover loaded with 10 ml of sterile 1 x PBS and placed on a rocking platform for 10 min. So that the eggs were washed completely to remove the bacteria on the egg shell surface. The inoculums were made in three dilutions viz. 10^{-1} , 10^{-2} and 10^{-3}

and depending upon the bacterial load, the dilutions were used for total bacterial count and the dilution factor was considered. The pour plate method was followed for taking the total bacterial count.

External and internal quality of table eggs during different storage period

The eggs were evaluated for external qualities in days of 1 (N=30; n=6), 7 (N=30; n=6), 14 (N=30; n=6) and 28 (N=30; n=6). External qualities of table eggs like egg shape index (SI), specific gravity (SG) and depth of air cell and internal quality viz., albumin index, yolk index and haugh unit measurement were calculated by using the standard procedure. All the data were analysed with One-Way ANOVA procedure using SPSS statistical package. The significant means were compared by Duncan's test.

Antibacterial effect of nano silver doped on table eggs

To assess the anti-bacterial effect of the test compounds in different concentrations along with positive (1% Kohrsolin) and negative control, individual colonies of different bacteria in different media viz. nutrient agar, MacConkey agar and anaerobic medium were isolated. From the isolated bacteria, 6 hrs fresh culture was prepared and adjusted to get $1-2 \times 10^6$ cfu/ml. Again, 100 μ l of this log phase bacterial culture was used to perform the antibacterial sensitivity test in the Muller Hinton agar plates against 10 ppm, 20 ppm and 50 ppm solutions of nano silver compound along with standard antibiotic (Cefotaxime 30 mcg), a positive control (1% Kohrsolin) and a negative control by the disc diffusion method. The discs were loaded with 100 μ l of each drug and negative control with PBS and further incubated for 24 hrs at 37°C.

RESULTS AND DISCUSSION

Nano silver doped egg shell surface viewed by Transmission Electron Microscope

Examination of nano silver on the egg shell membrane and its size is presented in plates 1, 2 and 3. The size of the nano silver in all the nano silver sprayed egg shell was ranged from 20 to 50 nm. The nano silver on the egg shell was scattered in lesser level in T_3 , and little more in T_4 , and comparatively highest in T_5 . The image of the nano silver in all the treated groups was very well appreciated.

Microbial count of the egg shell surface

The refrigeration storage condition had better bacterial load reduction with positive control and treatment groups when compared to room temperature condition (Table 2). Among the three treatment groups, 50 ppm nano silver treated group had numerically better bacterial reduction on the egg shell surface as compared to 10 and 20 ppm of the nano silver compounds. The nano silver (Ag-NP 124) compound at 50 ppm had 1-3 log reduction in the total bacterial count on one day after disinfection in the egg shell surface which were comparable with the positive control (1% Kohrsolin) used in the trial.

After 7 days of storage there was no significant difference in bacterial count between different treatment groups. Similar findings were observed by Banach *et al.* (2016) who found that eggs disinfected with silver nanoparticles amounted to $\times 10^4$ bacterial count on 30 min after disinfection and visible changes of total bacteria count on the 7th day of incubation and significant ($P < 0.05$) increase in the mean number of bacteria on the surface of all eggs on the 17th day of incubation.

Effect of different concentrations of nano silver on the external and internal qualities of table eggs

The external qualities viz, egg weight, shape index, specific gravity and air cell depth did not show any significant difference among the different treatments and control groups, when eggs were stored for 1, 7, 14 and 28 days at room and refrigeration temperatures (Table

Table 2. Effect of different concentrations of nano silver on microbial count of the egg shell surface

Storage Days	Storage condition									
	Room temperature (n=6)					Refrigeration temperature (n=6)				
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₁	T ₂	T ₃	T ₄	T ₅
1	3.4x10 ⁵	6.2x10 ⁴	0.9x10 ⁵	0.3x10 ⁵	6.7x10 ⁴	2.8x10 ⁶	2.6x10 ³	3.4x10 ⁵	2.2x10 ⁵	1.4x10 ⁴
7	2.4x10 ⁷	4.7x10 ⁵	3.1x10 ⁶	1.4x10 ⁵	1.5x10 ⁵	5.9x10 ⁶	6.8x10 ³	7.7x10 ⁵	4.6x10 ⁵	2.6x10 ⁴
14	3.3x10 ⁴	5.2x10 ³	7.0x10 ³	5.3x10 ³	3.3x10 ³	4.2x10 ⁷	0.6x10 ⁵	3.2x10 ⁶	3.5x10 ⁶	4.3x10 ⁵
21	3.8x10 ⁶	2.6x10 ⁴	1.4x10 ⁶	7.1x10 ⁵	8.4x10 ⁵	1.4x10 ⁸	7.3x10 ⁵	5.4x10 ⁷	5.8x10 ⁷	4.8x10 ⁶
28	5.7x10 ⁷	6.3x10 ⁶	2.6x10 ⁶	1.2x10 ⁷	5.2x10 ⁶	5.1x10 ⁸	2.5x10 ⁶	8.6x10 ⁷	5.4x10 ⁷	3.7x10 ⁶

3 and 4). Similarly, the internal qualities viz., albumin index, yolk index and Haugh unit showed no significant difference among the different treatments and control group eggs when eggs were stored for 1, 7, 14 and 28 days at room and refrigeration temperature (Table 5 and 6). Many researchers workers reported that nano silver may be used as a surface disinfectant, water disinfectant and therapeutic material in animal husbandry including poultry, livestock and aquatic industry (Nia, 2007 and Deshmukh *et al.*, 2019). Various diseases caused by bacteria, viruses, fungi and other mono-cellular microorganisms were effectively controlled by using nano silver compounds and it inhibits reproduction and growth of those bacteria and fungi responsible for the infection, bad odor, itchiness and sores (Korzeniowska *et al.*, 2015). Similarly, Viswanathan *et al.* (2016) reported that storing of eggs in the Ag NPs deposited in paper egg trays improved the shelf life of the eggs by more than 14 days compared to controls (eggs stored in conventional trays). On contrary, the present study clearly indicates that nano silver disinfection egg does not influence the external and internal quality characteristics of

eggs during different storage period in both room and refrigeration storage conditions.

Effect of different concentrations of nano silver on anti-bacterial activity (zone of inhibition)

Anti-bacterial activity testing with different bacteria against nano silver, standard antibiotic and controls showing zone of inhibition measurements (mm) in disc diffusion method is presented in Table 7. In disc diffusion method, the zone of inhibition was measured in mm and the standard antibiotic gave maximum zone of inhibition indicating complete clearance of the bacteria and the positive control showed better activity compared to the test compounds of nano silver at 10, 20 and 50 ppm levels against individual bacterial spp. However, nano silver 50 ppm had comparatively better zone of inhibition than 10 and 20 ppm levels (Plate 4). The isolated gram positive *Bacillus* and gram negative *E. coli* were presented in plate 5. Jung *et al.* (2008) observed that a reduction of more than 5 log (10) CFU/ml of both *S. aureus* and *E. coli* bacteria after 90 min of treatment with

Table 3. Effect of different concentrations of nano silver on the external quality of table eggs stored at room temperature (Mean $\pm$ SE) (n = 6)

Ext quality	1 day (N=30)							7 th day (N=30)						
	T ₁	T ₂	T ₃	T ₄	T ₅	F	Sig (NS)	T ₁	T ₂	T ₃	T ₄	T ₅	F	Sig (NS)
Egg Weight	57.56 $\pm$ 1.75	57.83 $\pm$ 1.23	60.26 $\pm$ 0.96	61.79 $\pm$ 1.08	58.22 $\pm$ 1.33	1.43	0.253	57.71 $\pm$ 1.13	59.48 $\pm$ 1.35	57.93 $\pm$ 1.38	55.36 $\pm$ 1.78	57.96 $\pm$ 1.31	1.097	0.380
Shape index	74.04 $\pm$ 1.40	75.22 $\pm$ 1.28	78.00 $\pm$ 1.48	76.21 $\pm$ 0.47	70.38 $\pm$ 1.31	1.184	0.342	75.32 $\pm$ 1.65	77.75 $\pm$ 1.69	77.70 $\pm$ 1.26	78.53 $\pm$ 1.07	77.36 $\pm$ 1.32	0.719	0.587
Specific gravity	1.04 $\pm$ 0.02	1.01 $\pm$ 0.02	1.03 $\pm$ 0.03	1.01 $\pm$ 0.02	1.01 $\pm$ 0.02	0.375	0.825	1.27 $\pm$ 0.02	1.30 $\pm$ 0.04	1.28 $\pm$ 0.03	1.22 $\pm$ 0.05	1.29 $\pm$ 0.03	0.555	0.697
air cell depth	0.36 $\pm$ 0.02	0.45 $\pm$ 0.04	0.43 $\pm$ 0.04	0.36 $\pm$ 0.03	0.35 $\pm$ 0.02	1.789	0.163	0.36 $\pm$ 0.03	0.35 $\pm$ 0.02	0.35 $\pm$ 0.02	0.46 $\pm$ 0.03	0.38 $\pm$ 0.03	2.833	0.146

14 th day (N=30)							28 th day (N=30)						
T ₁	T ₂	T ₃	T ₄	T ₅	F	Sig (NS)	T ₁	T ₂	T ₃	T ₄	T ₅	F	Sig (NS)
58.3 $\pm$ 0.98	60.70 $\pm$ 1.66	55.63 $\pm$ 2.44	60.00 $\pm$ 1.12	57.81 $\pm$ 1.56	1.470	0.241	57.91 $\pm$ 0.71	57.18 $\pm$ 1.33	58.96 $\pm$ 1.56	57.28 $\pm$ 1.08	56.30 $\pm$ 1.74	1.621	0.200
77.37 $\pm$ 1.66	79.14 $\pm$ 0.61	77.19 $\pm$ 0.82	79.58 $\pm$ 0.26	80.55 $\pm$ 0.52	2.464	0.171	79.90 $\pm$ 0.74	77.64 $\pm$ 1.02	77.29 $\pm$ 0.80	78.02 $\pm$ 1.07	79.96 $\pm$ 1.56	1.385	0.268
1.04 $\pm$ 0.04	1.13 $\pm$ 0.07	1.06 $\pm$ 0.06	1.06 $\pm$ 0.03	0.99 $\pm$ 0.02	0.938	0.458	1.08 $\pm$ 0.04	1.02 $\pm$ 0.04	1.04 $\pm$ 0.03	1.02 $\pm$ 0.03	1.11 $\pm$ 0.03	0.881	0.489
0.35 $\pm$ 0.02	0.38 $\pm$ 0.04	0.40 $\pm$ 0.02	0.38 $\pm$ 0.04	0.40 $\pm$ 0.02	0.364	0.832	0.41 $\pm$ 0.05	0.41 $\pm$ 0.04	0.43 $\pm$ 0.04	0.51 $\pm$ 0.07	0.45 $\pm$ 0.05	0.524	0.719

NS-Non significant (P>0.05)

Table 4. Effect of different concentrations of nano silver on the external quality of table eggs stored at refrigeration temperature (Mean $\pm$ SE) (n = 6)

Ext quality	1 day (N=30)							7 th day (N=30)						
	T ₁	T ₂	T ₃	T ₄	T ₅	F	Sig (NS)	T ₁	T ₂	T ₃	T ₄	T ₅	F	Sig (NS)
Egg Weight	57.93 $\pm$ 0.36	57.92 $\pm$ 0.34	60.68 $\pm$ 1.04	62.30 $\pm$ 1.93	60.42 $\pm$ 0.85	3.107	0.335	58.05 $\pm$ 1.93	57.88 $\pm$ 1.36	57.80 $\pm$ 1.87	57.20 $\pm$ 1.14	56.85 $\pm$ 1.29	0.107	0.979
Shape index	77.97 $\pm$ 0.97	75.70 $\pm$ 0.82	74.26 $\pm$ 0.76	77.96 $\pm$ 2.90	75.58 $\pm$ 2.15	0.866	0.498	76.48 $\pm$ 1.70	78.33 $\pm$ 0.97	76.26 $\pm$ 1.32	77.44 $\pm$ 0.82	77.4 $\pm$ 1.61	0.394	0.811
Specific gravity	1.11 $\pm$ 0.02	1.05 $\pm$ 0.03	1.07 $\pm$ 0.03	1.07 $\pm$ 0.01	1.07 $\pm$ 0.02	0.757	0.563	1.11 $\pm$ 0.04	1.09 $\pm$ 0.02	1.20 $\pm$ 0.04	1.08 $\pm$ 0.04	1.08 $\pm$ 0.03	1.496	0.233
Aircell depth	0.41 $\pm$ 0.03	0.36 $\pm$ 0.02	0.36 $\pm$ 0.03	0.36 $\pm$ 0.03	0.36 $\pm$ 0.03	0.529	0.715	0.38 $\pm$ 0.03	0.40 $\pm$ 0.05	0.50 $\pm$ 0.10	0.36 $\pm$ 0.03	0.36 $\pm$ 0.04	0.841	0.512

14 th day (N=30)							28 th day (N=30)						
T ₁	T ₂	T ₃	T ₄	T ₅	F	Sig (NS)	T ₁	T ₂	T ₃	T ₄	T ₅	F	Sig (NS)
58.41 $\pm$ 1.28	58.98 $\pm$ 0.60	57.96 $\pm$ 1.01	58.91 $\pm$ 1.06	55.40 $\pm$ 1.21	1.925	0.137	54.66 $\pm$ 0.79	57.75 $\pm$ 1.75	58.08 $\pm$ 2.58	57.00 $\pm$ 2.06	54.68 $\pm$ 1.06	0.81	0.52
79.15 $\pm$ 0.76	79.09 $\pm$ 1.01	78.61 $\pm$ 1.01	79.93 $\pm$ 1.06	81.70 $\pm$ 1.23	1.392	0.265	78.34 $\pm$ 1.43	78.55 $\pm$ 1.09	79.37 $\pm$ 1.24	80.93 $\pm$ 1.35	80.47 $\pm$ 0.79	0.90	0.47
1.02 $\pm$ 0.04	1.04 $\pm$ 0.02	1.04 $\pm$ 0.04	1.10 $\pm$ 0.04	0.97 $\pm$ 0.02	1.426	0.254	0.99 $\pm$ 0.01	1.03 $\pm$ 0.03	1.00 $\pm$ 0.06	0.99 $\pm$ 0.05	0.77 $\pm$ 0.15	1.735	0.174
0.48 $\pm$ 0.03	0.58 $\pm$ 0.06	0.48 $\pm$ 0.04	0.50 $\pm$ 0.04	0.58 $\pm$ 0.05	1.156	0.354	0.50 $\pm$ 0.06	0.53 $\pm$ 0.06	0.60 $\pm$ 0.04	0.53 $\pm$ 0.02	0.55 $\pm$ 0.05	0.498	0.737

NS-Non significant (P > 0.05)

Table 5. Effect of different concentrations of nano silver on the internal quality of table eggs stored at room temperature (Mean $\pm$ SE) (n = 6)

Int. quality	1 day (N=30)							7 th day (N=30)						
	T ₁	T ₂	T ₃	T ₄	T ₅	F	Sig (NS)	T ₁	T ₂	T ₃	T ₄	T ₅	F	Sig (NS)
Albumin Index	5.085 $\pm$ 0.36	3.45 $\pm$ 0.56	3.94 $\pm$ 0.66	4.29 $\pm$ 0.52	4.48 $\pm$ 0.52	1.279	0.305	1.39 $\pm$ 0.15	1.41 $\pm$ 0.21	1.56 $\pm$ 0.11	1.30 $\pm$ 0.13	1.30 $\pm$ 0.14	0.487	0.745
Yolk Index	33.99 $\pm$ 1.48	34.09 $\pm$ 1.16	32.10 $\pm$ 1.17	34.98 $\pm$ 2.05	34.61 $\pm$ 2.20	0.441	0.778	30.10 $\pm$ 1.62	30.50 $\pm$ 1.02	30.15 $\pm$ 0.94	29.79 $\pm$ 1.22	26.76 $\pm$ 1.38	1.468	0.242
Haugh Unit	59.62 $\pm$ 2.77	53.29 $\pm$ 5.90	54.76 $\pm$ 13.74	58.05 $\pm$ 5.45	61.41 $\pm$ 4.89	0.742	0.573	21.52 $\pm$ 5.06	17.17 $\pm$ 7.52	26.05 $\pm$ 3.56	20.70 $\pm$ 5.58	15.56 $\pm$ 6.95	0.480	0.750

14 th day (N=30)							28 th day (N=30)						
T ₁	T ₂	T ₃	T ₄	T ₅	F	Sig (NS)	T ₁	T ₂	T ₃	T ₄	T ₅	F	Sig (NS)
1.23 $\pm$ 0.16	1.24 $\pm$ 0.06	1.50 $\pm$ 0.05	1.26 $\pm$ 0.08	1.23 $\pm$ 0.15	1.039	0.407	1.46 $\pm$ 0.10	1.34 $\pm$ 0.12	1.23 $\pm$ 0.10	1.24 $\pm$ 0.09	1.51 $\pm$ 0.05	1.583	0.210
25.41 $\pm$ 2.18	25.78 $\pm$ 1.55	26.32 $\pm$ 1.05	26.22 $\pm$ 1.82	26.97 $\pm$ 1.40	0.127	0.971	25.36 $\pm$ 1.54	26.39 $\pm$ 1.25	25.93 $\pm$ 1.47	27.19 $\pm$ 1.63	25.68 $\pm$ 1.86	0.205	0.933
17.95 $\pm$ 5.16	18.74 $\pm$ 2.55	31.97 $\pm$ 3.57	20.53 $\pm$ 4.76	23.29 $\pm$ 4.64	1.786	0.163	25.70 $\pm$ 3.40	24.25 $\pm$ 5.74	20.96 $\pm$ 4.79	22.72 $\pm$ 3.59	28.39 $\pm$ 0.99	0.495	0.739

NS-Non significant (P > 0.05)

Table 6. Effect of different concentrations of nano silver on the internal quality of table eggs stored at refrigeration temperature (Mean $\pm$ SE) (n = 6)

Int. quality	1 day (N=30)							7 th day (N=30)						
	T ₁	T ₂	T ₃	T ₄	T ₅	F	Sig (NS)	T ₁	T ₂	T ₃	T ₄	T ₅	F	Sig (NS)
Albumin Index	4.70 $\pm$ 0.54	3.80 $\pm$ 0.67	4.27 $\pm$ 0.35	3.50 $\pm$ 0.34	3.99 $\pm$ 0.29	0.968	0.442	3.89 $\pm$ 0.40	4.07 $\pm$ 0.06	3.79 $\pm$ 0.27	3.84 $\pm$ 0.54	3.41 $\pm$ 0.49	0.378	0.822
Yolk Index	36.42 $\pm$ 1.51	34.19 $\pm$ 1.23	33.32 $\pm$ 1.15	36.01 $\pm$ 1.27	37.61 $\pm$ 1.47	1.681	0.186	26.73 $\pm$ 1.11	28.23 $\pm$ 0.93	28.83 $\pm$ 0.85	26.95 $\pm$ 0.99	25.96 $\pm$ 1.28	1.238	0.320
Haugh Unit	51.21 $\pm$ 4.13	54.04 $\pm$ 7.99	58.64 $\pm$ 3.62	48.01 $\pm$ 3.74	56.66 $\pm$ 2.97	1.088	0.384	46.78 $\pm$ 3.94	49.70 $\pm$ 1.80	47.30 $\pm$ 3.69	43.99 $\pm$ 7.11	41.24 $\pm$ 7.12	0.396	0.809

14 th day (N=30)							28 th day (N=30)						
T ₁	T ₂	T ₃	T ₄	T ₅	F	Sig (NS)	T ₁	T ₂	T ₃	T ₄	T ₅	F	Sig (NS)
2.87 $\pm$ 0.25	2.87 $\pm$ 0.31	3.15 $\pm$ 0.33	3.70 $\pm$ 0.93	3.01 $\pm$ 0.38	0.463	0.762	2.89 $\pm$ 0.16	2.50 $\pm$.30	2.56 $\pm$ 0.19	2.51 $\pm$ 0.14	2.31 $\pm$ 0.26	0.86	0.490
27.74 $\pm$ 1.56	28.15 $\pm$ 1.90	32.09 $\pm$ 1.44	30.62 $\pm$ 3.03	28.76 $\pm$ 0.87	0.927	0.464	26.90 $\pm$ 1.74	27.84 $\pm$ 0.54	27.89 $\pm$ 1.38	27.34 $\pm$ 1.01	28.36 $\pm$ 1.06	0.210	0.931
46.50 $\pm$ 2.68	46.08 $\pm$ 3.96	49.72 $\pm$ 3.72	52.36 $\pm$ 7.13	49.14 $\pm$ 4.76	0.298	0.876	51.74 $\pm$ 2.96	42.58 $\pm$ 6.02	46.29 $\pm$ 4.23	45.53 $\pm$ 4.26	52.58 $\pm$ 10.56	0.473	0.755

NS-Non significant (P >0.05)

Table 7. Anti-bacterial activity (zone of inhibition) with different bacteria against nano silver, standard antibiotic and controls in disc diffusion method

S.No	Bacterial Sp.	Zone of inhibition (mm)					
		T ₁	T ₂	T ₃	T ₄	T ₅	Antibiotic Cefotaxime CTX-30mcg
1	<i>Bacillus sp</i>	0	6	2	4	4	30
2	<i>Staphylococcus spp</i>	0	22	2	4	6	24
3	<i>Clostridium spp</i>	0	16	0	6	6	28
4	<i>Escherichia coli</i>	0	14	0	2	4	14
5	Mixed (all above)	0	4	0	4	4	8

the silver ion solution. Similarly, Ru Li *et al.* (2010) reported that 10 µg/ml silver nano particles could completely inhibit the growth of *E. coli* within 7 days and concluded that minimum inhibitory concentration (MIC) of silver nano particles to *E. coli* was 10 µg/ml. Studies proved that nano silver with a diameter of 22.5 nm increase the antibacterial activity of some antibiotics, such as penicillin G, amoxicillin, erythromycin, clindamycin, and vancomycin (Shahverdi *et al.*, 2007).

It indicates that nano silver compounds resulted in the leakage of reducing sugars and proteins and induced the respiratory chain dehydrogenases into inactive state, suggesting that nano silver were able to destroy the permeability of the bacterial membranes. In addition, silver ion released from nano silver interacts with phosphorus in DNA as well as with sulphur containing proteins resulted into inhibition of enzymes activities (Deshmukh *et al.*, 2019). Particle size and shape are also other

parameters to determine the antimicrobial activity (Morones *et al.*, 2005).

It was concluded that the eggs sprayed with nano silver stored for 1, 7, 14 and 28 days in room and refrigeration temperatures showed no significant difference in external and internal qualities of table eggs. However, it was evident that the nano silver (Ag-NP124) compound at 50 ppm had 1-3 log reduction in the total bacterial count on the egg shell surface. However, a further study may be recommended to find out the antimicrobial property with higher concentration of nano silver to use as egg shell sanitizer for commercial purpose.

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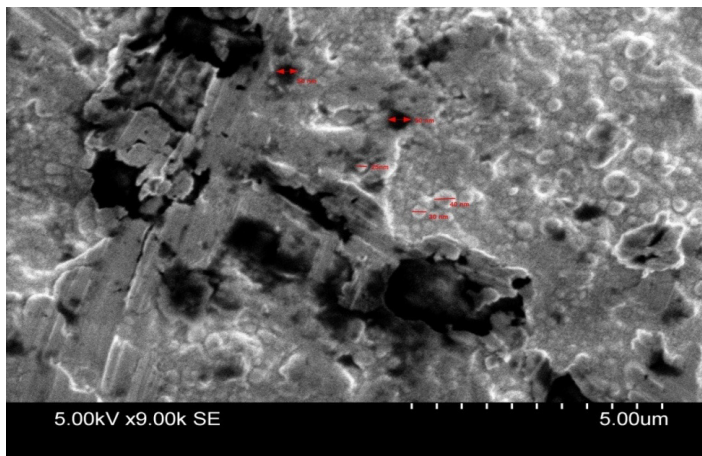


Plate 1 - Nano silver image in 10 ppm

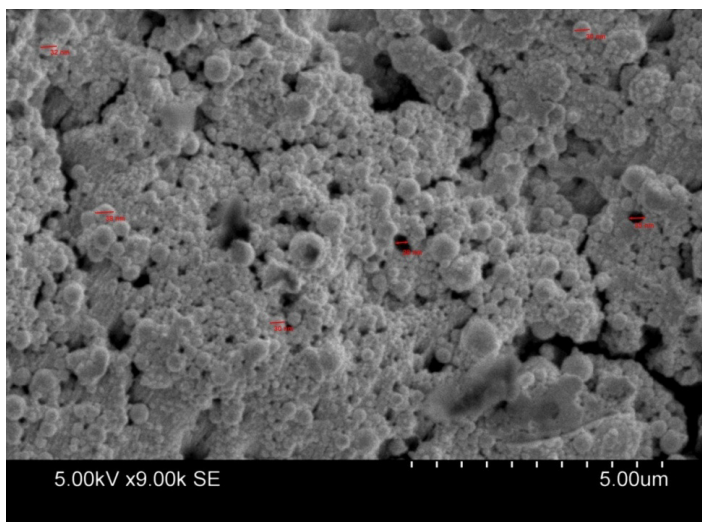


Plate 2 - Nano silver image in 20 ppm

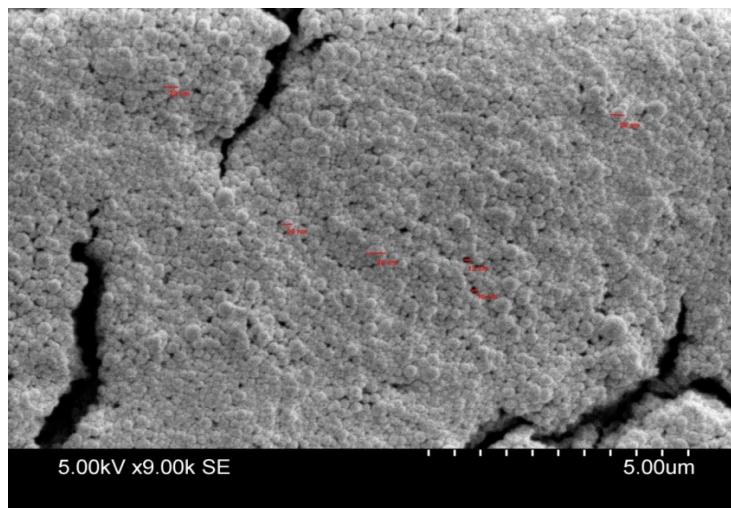


Plate 3 - Nano silver image in 50 ppm

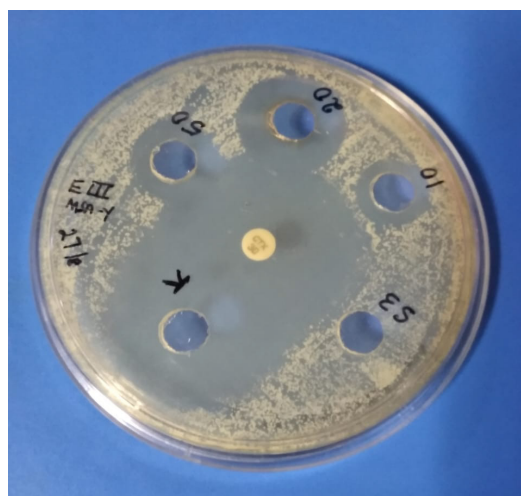
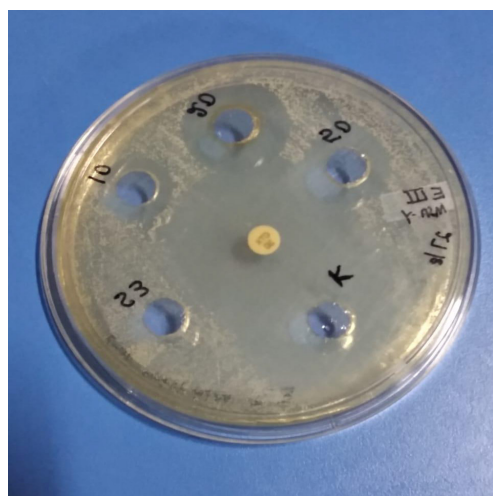
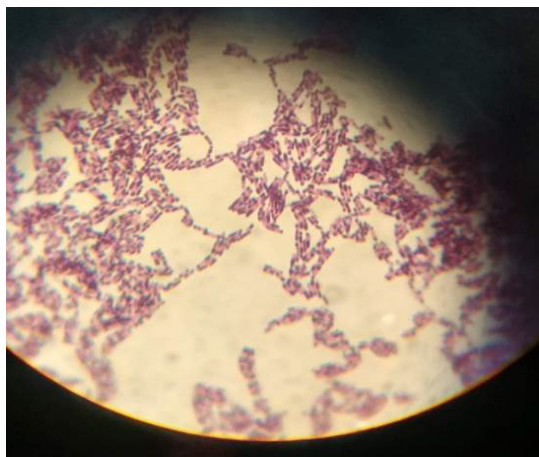
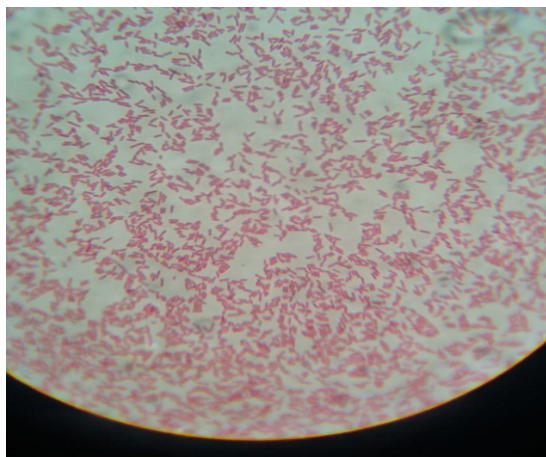


Plate 4. Muller Hinton plate showing Zone of Inhibition (ZOI) for the bacteria isolated from egg shell surface using standard antibiotic disc and nano silver solution



Gram positive *Bacillus* species



Gram negative *E. coli*

Plate 5. Gram positive and gram negative bacteria isolated from egg shell surface