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Research Article

Development & Evaluation of Hydrogen Peroxide and Silver Nitrate Disinfection Solution

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ABSTRACT

This study presents the formulation, characterization, and efficacy evaluation of a novel disinfectant solution combining hydrogen peroxide (11%) and silver nitrate (100–500 ppm), stabilized with aromatic amide compounds to enhance chemical stability and antimicrobial performance. Hydrogen peroxide and silver nitrate were selected for their strong oxidizing and broad-spectrum antimicrobial properties. Preformulation analyses confirmed high-purity reagents, and pH assessments revealed increasing acidity with higher active concentrations. Initial screening of six formulations demonstrated significant synergistic antimicrobial activity, achieving up to a 6-log microbial reduction. Optimized batches were characterized for clarity, particle size, zeta potential, and antimicrobial efficacy, with higher silver concentrations yielding better inhibition zones. Comparative analysis of two optimized batches (Batch-B: 300 ppm $AgNO_3$; Batch-C: 500 ppm $AgNO_3$) revealed that Batch-C exhibited superior antimicrobial activity and particle uniformity, whereas Batch-B offered better colloidal stability. In-vitro fogging studies demonstrated that both formulations effectively reduced airborne microbial loads, with the 500-ppm formulation maintaining lower microbial counts over time. These findings support the use of hydrogen peroxide and silver nanoparticle-based disinfectants for effective, short-term disinfection in enclosed environments, offering a safer alternative to traditional agents like formaldehyde.

INTRODUCTION

The method of eliminating microorganisms from inanimate object surfaces is known as disinfection and chemical used for disinfection are called as disinfectants. Disinfectants are designed for use to eradicate or stop the spread of bacteria of any type because of their antimicrobial action. These are sometimes referred to as bactericides, are used in laboratories and hospitals to avoid infections importantly in hospital environments due to pathogens living on hospital surfaces being the

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direct cause for hospital-acquired infections. The two main uses of chemical disinfectants be water-related disinfection and surface sterilization. Chlorine is the most commonly employed disinfectant to sanitize drinking water. For surface disinfection peracetic acid, glutaraldehyde, formaldehyde, iodide and iodiophore sanitation, alcohol, phenols, hydrogen peroxide, hypochlorite, etc. are used. Glutaraldehyde and peracetic acid are widely applied to sterilize environmental surfaces and medical devices. Disinfecting agents are necessary to prevent potential contamination when cleaning tools as well as workplace appears specifically in labs whereas researchers examine a large number of organisms tests [1]. Hospital treatment necessitates the highest level of sanitation since medical gadgets and surgical instruments, such as implants, come into close touch with bodily fluids. Poor equipment hygiene can result in mortality or irreparable infections in immunocompromised individuals. As a result, it is critical to use a disinfectant that is successful not only in removing bacteria swiftly and reliably, but also in preventing harm to the patient's health and eliminating unpleasant odors. Reducing microbiological contamination in the hospital setting is an important part of an infection prevention strategy. This is particularly true in an era of greater attention on hospital-acquired illnesses and multidrug-resistant pathogens. Moreover because of its simplicity of use as well as a growing number of studies demonstrating its effectiveness, hydrogen peroxide-based automatic room disinfecting devices are being used more frequently in pharmaceuticals and healthcare settings. Hydrogen peroxide (H_2O_2) and silver ions, shows synergistic strong bactericidal activities [2]. The experiment's goal was to determine how well different concentration ratios of Hydrogen peroxide (H_2O_2) and silver ions disinfectants performed against different air born micro-organisms in the particular area [3]. In hospitals, hydrogen peroxide is often used alongside chlorine-based solutions and quaternary ammonium compounds as an effective antibacterial agent for cleaning and disinfecting. It can eliminate a wide range of microbes, including yeast, bacterial fungi, and infectious diseases. While hydrogen peroxide is generally believed to damage cells due to its strong oxidizing nature, it is understood that at appropriate concentrations, it is essential for effective wound healing. Proper application of hydrogen peroxide can improve recovery outcomes by not only removing harmful bacteria but also by playing a regulatory role in the healing process. Its current use in medicine is primarily limited to pathogen removal and occasionally to blood clotting, though deeper understanding of its effects could lead to broader applications in recovery. Since the beginning of the 20th century, silver has been applied as an antibacterial agent to cure illnesses. In particular, at high concentrations of substances, silver nitrate has been indicated to have a variety of impacts on microorganisms including reducing protein thiol groups, leading to genetic material to be in the compressed state rather than the relaxed form, which prevents the replication of cells, and leading to cell death that is programmed (cell destruction). Bacteria can synthesize silver nanoparticles, but only at low quantities [4]. Silver, nitrogen, and oxygen combine to form the inorganic chemical known as silver nitrate ($AgNO_3$). It is highly soluble in water and appears as a white, crystallized substance. Silver nitrate is commonly used in various fields, including photography, medicine, and chemical synthesis. In photography, it is essential for the production of photographic films and papers. In medicine, silver nitrate serves as an antiseptic, particularly for treating wounds and burns due to its antimicrobial properties. It is also used in cauterization and the removal of warts or unwanted tissues. Silver nitrate is a reagent used

in labs to prepare other silver compounds and detect halides. It must be handled carefully, though, because it is caustic and can discolor flesh or clothing. Fumigation of a room using disinfectants is a process designed to sanitize and eliminate harmful microorganisms, such as bacteria, viruses, and fungi, from the air and surfaces within the space. In this method, a disinfectant is aerosolized or vaporized and released into the room, allowing it to settle on various surfaces and in the air, effectively killing germs and bacteria. The room is typically sealed during the fumigation process to ensure the disinfectant reaches all areas, including hidden or hard-to-reach spots. The fumigation process is often used in hospitals, offices, kitchens, and other areas that require a high level of cleanliness. [5]. Fumigation is also used in other sectors, including food, pharmaceuticals, and public health. It is effective for disinfecting not only surfaces but also air in enclosed spaces such as medical laboratories, incubation units, refrigerators, freezers, vehicles, trains, and airplanes [6]. Additionally, it plays a crucial role in controlling airborne diseases. Pathogens can remain viable on surfaces for extended periods and are transmitted via respiratory droplets and hand contact. After touching contaminated surfaces, individuals may transfer pathogens to their nose, mouth, or eyes, contributing to the spread of infection. Fumigation helps break this chain by disinfecting both air and surfaces, especially in high-risk areas such as hospitals and public parks [7].

2.0. MATERIALS AND METHODS

2.1. Materials

Certain excipients and chemicals were used in the preparation of hydrogen peroxide and silver nitrate disinfection solution. List of different excipients and chemicals used in this study is given in table no. 2.1.

Table. 2.1: List of ingredients and chemicals included in the current study

Sr. No.	Name Of Ingredients	Manufacturer
1.	Hydrogen Peroxide	Novetra. Medicare LLP, Tasgaon Dist- Sangli. Maharashra
2.	Silver Nitrate AR	Research-Lab Fine chem Pvt. Ltd., Mumbai
3.	Potassium permanganate	Loba chemie Pvt. Ltd., Mumbai
4.	Sulphuric acid	Loba chemie Pvt. Ltd., Mumbai
5.	Oxalic acid	Loba chemie Pvt. Ltd., Mumbai
6.	Phenolphthalein indicator	Loba chemie Pvt. Ltd., Mumbai
7.	Sodium Chloride	Loba chemie Pvt. Ltd., Mumbai
8.	Potassium chromate	Research-Lab Fine chem Pvt. Ltd., Mumbai
9.	Sulphuric acid	Loba chemie Pvt. Ltd., Mumbai
10.	Ethanol	Loba chemie Pvt. Ltd., Mumbai
11.	Acetanilide	Research-Lab Fine chem Pvt. Ltd., Mumbai
12.	Aromatic Amide compounds	Research-Lab Fine chem Pvt. Ltd., Mumbai
13.	N-phenyl aminobenzene	Loba chemie Pvt. Ltd., Mumbai
14.	Benzoic acid	Loba chemie Pvt. Ltd., Mumbai
15.	Methanol	Loba chemie Pvt. Ltd., Mumbai

A. Composition:

- **Hydrogen Peroxide**: 20%–75% by weight (preferably 3%–11%).
- **Stabilizer (AAC)**: 0.01 to 7 mg/kg, preferably 1–2.5 mg/kg. Examples include acetanilide, N-phenyl aminobenzene, and benzoic acid derivatives.
- **Silver Nitrate ($AgNO_3$)**: 0.002% to 1.0% w/v (preferably 0.05%–0.5%) to generate colloidal nano silver in situ.

B. Preparation Method:

The following method was adopted for preparation:

1. **Mixing**: In a dust-free HDPE vessel, concentrated H_2O_2 (3%–11%) was blended with the selected AAC stabilizer (0.1–2%) and $AgNO_3$ (100–500 ppm).

2. **Nano-Silver Generation**: The system was stirred at 10–50 RPM and maintained at 25–35°C for 4 hours. The in-situ reaction led to the formation of nano-silver particles stabilized by AAC.

3. **Post-processing**: The mixture was optionally filtered to remove particulates or insoluble residues.

2.2. In-vitro Performance Evaluation/Efficacy study:

To assess the efficacy of a disinfectant solution in reducing airborne microbial contamination through the fogging method by comparing microbial counts before and after application in a controlled environment.

2.2.1. Selection and measurement of Test Environment

A controlled, enclosed chamber or room which is the area was calculated with the help of meter tape. These meter tape measures the total volume of room including length, width as well as height.

2.2.2. Microbial Sampling Before and After Fogging:

A. Air Sampling Techniques:

- **Settle Plate Method:**

The **Settle Plate Method** is a passive air sampling technique used to assess microbial contamination in controlled environments such as clean rooms, hospitals, pharmaceutical manufacturing areas, and food processing facilities [8]. This method relies on the natural settling of airborne microorganisms onto the surface of an exposed agar plate over a specific period, typically ranging from 30 minutes to 4 hours.

B. Preparation and Incubation of different agar for bacterial growth:

The preparation and incubation of Nutrient Agar, Blood Agar, MacConkey Agar, and Sabouroud Dextrose-Chloramphenicol Agar follow standard microbiological procedures to support bacterial growth. Nutrient Agar is prepared by dissolving the dehydrated medium in distilled water, autoclaving at 121°C for 15 minutes, and pouring it into sterile Petri dishes, followed by incubation at 30-37°C for 24-48 hours after inoculation. The generally used decontamination technique, hot water sterilization (autoclave), is thought to be a trusted and affordable process for sterilizing healthcare equipment [9].

C. Fogging: A fogger machine was used to analyze data before and after application:

A fogger is used for selected room fogging to disinfect and decontaminate specific areas, such as hospital isolation rooms, pharmaceutical clean rooms, laboratories, and food processing units, where microbial control is essential. The fogger disperses a fine mist of disinfectant or antimicrobial agent, allowing it to reach surfaces, air, and hard-to-access areas, ensuring thorough sterilization. Fogging is a process in which disinfectant is spray in the air to kill those microorganisms which are present in environment [10].

D. Data Analysis

- **Microbial Count Before vs. After Fogging:**

Microbial count before and after fogging can be accurately measured using a colony counter

machine, which automates the counting of colony-forming units (CFU) on agar plates. Before fogging, air samples are collected using air samplers or settle plates, which are incubated under appropriate conditions. During studies and examinations, computerized colony counts' precision and practicality are significant [11]. Colony that established themselves are often countable and clearly apparent with a normal vision. Hence, the number of microbes within the initial solution are inversely expressed through the word CFU, or usually CFU/millilitre [12].

Aim And Objectives

3.1.1 Aim:

"To develop and evaluate a hydrogen peroxide and silver nitrate-based aerial disinfection solution for effective control of airborne microbial contaminants through optimization of formulation, characterization, and performance assessment."

3.1.2 Objectives of Work:

1. To develop and optimize hydrogen peroxide conjugated silver nitrate solution for aerial disinfection purpose.

2. To evaluate hydrogen peroxide conjugated silver nitrate solution with commercial samples in suitable in-vitro methods for generating efficacy data for aerial disinfection.

4.0. Development And Optimization of Preliminary Trial Disinfection Solution Batches Containing Hydrogen Peroxide and Silver Nitrate.

4.1 Development of Preliminary Trial Disinfection Solution Batches Disinfection Solution Batches Containing Hydrogen Peroxide and Silver Nitrate.

Investigate impact of altering Amount of Hydrogen peroxide (ml), Concentration of Silver Nitrate (PPM), Percentage of Hydrogen peroxide, and to characterize silver nanoparticles, initial trial batches of hydrogen peroxide and silver nitrate disinfection solution, were prepared. All disinfectant trial batches analysed by its visual appearance, size of particles, zeta potential, Hydrogen peroxide and silver nitrate disinfection solution were prepared by using conjunction method. Initially various hydrogen peroxide percentages (v/v), such as 3%, 6%, and 11% (v/v). As well as silver nitrate concentration varies like 100 ppm, 300 ppm and 500ppm. They mixing with each other's, under magnetic stirrer at rpm of 500 to get clear phase solution. The resulting mixture was transferred, while stirring constantly, into a small in Beaker filling with 100 millilitres. Formulation will be prepared.

4.2 Analysis of Preliminary Trial Disinfection Solution Batches Containing Hydrogen Peroxide and Silver Nitrate.

Preliminary trial batches of the hydrogen peroxide and silver nitrate-based disinfection solution were evaluated for antimicrobial efficacy using the **zone of inhibition method**. The results demonstrated clear and measurable zones of inhibition against selected bacterial strains, indicating effective antimicrobial activity. Variations in zone size were observed depending on the silver nitrate concentration, with higher concentrations generally producing larger zones, suggesting enhanced synergistic action with hydrogen peroxide. These findings support the formulation's potential as a broad-spectrum disinfectant and provide a basis for further optimization and validation studies

4.3 Optimisation of Disinfection Solution Batches Containing Hydrogen Peroxide and Silver Nitrate.

To optimize the stability and efficacy of the silver Nitrate and hydrogen peroxide formulation, a series of developmental batches were prepared at laboratory scale. Each batch was designed to explore the influence of stabilizer concentration, silver nitrate level, and hydrogen peroxide strength on the final product characteristics, particularly chemical stability, silver particle formation, and antimicrobial potential. The batches were formulated using a controlled process in high-density polyethylene (HDPE) vessels with low-shear mixing (10–50 RPM) at ambient temperature (25–35°C).

Table.4.1. Three batches Disinfection Solution Batches Containing Hydrogen Peroxide and Silver Nitrate.

Component	Batch A	Batch B	Batch C
Hydrogen Peroxide (w/w %)	11.00%	11.00%	11.00%
Aromatic Amide Stabilizer (mg/kg)	1.0 mg/kg	2.0 mg/kg	2.5 mg/kg
Silver Nitrate (ppm)	100 ppm	300 ppm	500 ppm
Water (q.s.)	Up to 100%	Up to 100%	Up to 100%

Three disinfectant formulations (Batch A, B, and C) were developed containing a fixed concentration of Hydrogen Peroxide (11% v/v) as the primary active agent. To enhance stability, varying concentrations of an aromatic amide stabilizer were incorporated: 1.0 mg/kg in Batch A, 2.0 mg/kg in Batch B, and 2.5 mg/kg in Batch C. Additionally, Silver Nitrate was added as a secondary antimicrobial agent at 100 ppm (Batch A), 300 ppm (Batch B), and 500 ppm (Batch C). Each formulation was prepared using Water to make up the final volume to 100%. The components were mixed under controlled conditions to ensure homogeneity and clarity.

4.4. Optimized Final Batch of Disinfection Solution Containing Hydrogen Peroxide and Silver Nitrate.

After preparation, all batches were analyzed for hydrogen peroxide assay, particle size, zeta potential, and polydispersity index using validated analytical methods. In contrast in the preliminary stage, we run three batches of disinfection solutions containing hydrogen peroxide and silver nitrate.

4.5. Formulation of comparative batches hydrogen peroxide and silver nitrate disinfection solution performing In-vitro Efficacy study.

300 ppm as well as 500 ppm of silver ions generated by the AgNO3 conjunction and 11% hydrogen peroxide (Merck's) served as sample preparations. According to Pedahzur R et al.,[13], a cleansing mixture using 11% H2O2 and 300 ppm Ag+ originated just getting within ionised water, using experimentally determined improvements.

Table. 4.2. Final Two Disinfection Solution Batches Containing Hydrogen Peroxide and Silver Nitrate.

Formulation batches	Hydrogen peroxide (%)	Silver nitrate (ppm)	Preparation of disinfection solution (ml)
Batch-B	11	300	100
Batch-C	11	500	100

5.0. RESULTS AND DISCUSSION

5.1. Development and Analysis of Preliminary Trial Batches Hydrogen Peroxide and Silver Nitrate-Based Disinfection Solution

A. Development of Preliminary Trial Hydrogen Peroxide and Silver Nitrate-Based Disinfection Solution

We formulated hydrogen peroxide eight batches H-1 to H-8 as well as silver Nitrate four batches S-1 to S-4 are as follows in table.8.4.

Table.5.1. Composition of Hydrogen Peroxide Percentage and Silver Nitrate.

A) Hydrogen Peroxide percentage	Preliminary trial batches	Preparation Of disinfectant (ml)	Percentage of Hydrogen peroxide (%v/v)	Concentration of Silver Nitrate (PPM)
	H-1	100	3%	300ppm
	H-2	100	6%	300ppm
	H-3	100	9%	300ppm
	H-4	100	11%	300ppm
	H-5	100	3%	500ppm
	H-6	100	6%	500ppm
	H-7	100	9%	500ppm
	H-8	100	11%	500ppm
B) Silver nitrate	S-1	100	11%	100 ppm
	S-2	100	11%	300ppm
	S-3	100	11%	100 ppm
	S-4	100	11%	500ppm

B. Analysis of Preliminary Trial Batches Hydrogen Peroxide and Silver Nitrate-Based Disinfection Solution

Preliminary trial batches of the hydrogen peroxide and silver nitrate-based disinfection solution were evaluated for antimicrobial efficacy using the zone of inhibition method. The results demonstrated clear and measurable zones of inhibition against selected bacterial strains, indicating effective antimicrobial activity. Variations in zone size were observed depending on the silver nitrate concentration, with higher concentrations generally producing larger zones, suggesting enhanced synergistic action with hydrogen peroxide. These findings support the formulation's potential as a broad-spectrum disinfectant and provide a basis for further optimization and validation studies. The provided data highlights the antimicrobial efficacy of hydrogen peroxide (H_2O_2) and silver nitrate ($AgNO_3$) combinations microbial pathogens. The study measured two key indicators: the Zone of Inhibition (ZOI), indicating the area where bacterial growth is prevented, and the Log Reduction, representing the decrease in viable bacterial count.

Table No.5.2. Observation table of Zone of Inhibition (ZOI) for Preliminary Trial Batches

Sr. No	H_2O_2 Concentration (%)	$AgNO_3$ Concentration (ppm)	Zone of Inhibition (mm)	Log Reduction	Interpretation
1	3%	0	12	3	Moderate effect

2	6%	0	15	3.5	Increased effect with higher H_2O_2
3	9%	0	17	4	Stronger effect
4	11%	0	18	4.3	High H_2O_2 shows good antimicrobial action
5	3%	100 ppm	14	3.5	Slight improvement over H_2O_2 alone
6	3%	300 ppm	17	4.5	Significant enhancement
7	3%	500 ppm	20	5	Synergistic effect clearly observed
8	11%	300 ppm	23	5.5	Strong synergy with higher H_2O_2 and $AgNO_3$
9	11%	500 ppm	25	6	Maximum observed antimicrobial effect
10	—	Commercial disinfectant	20	5	Benchmark comparison

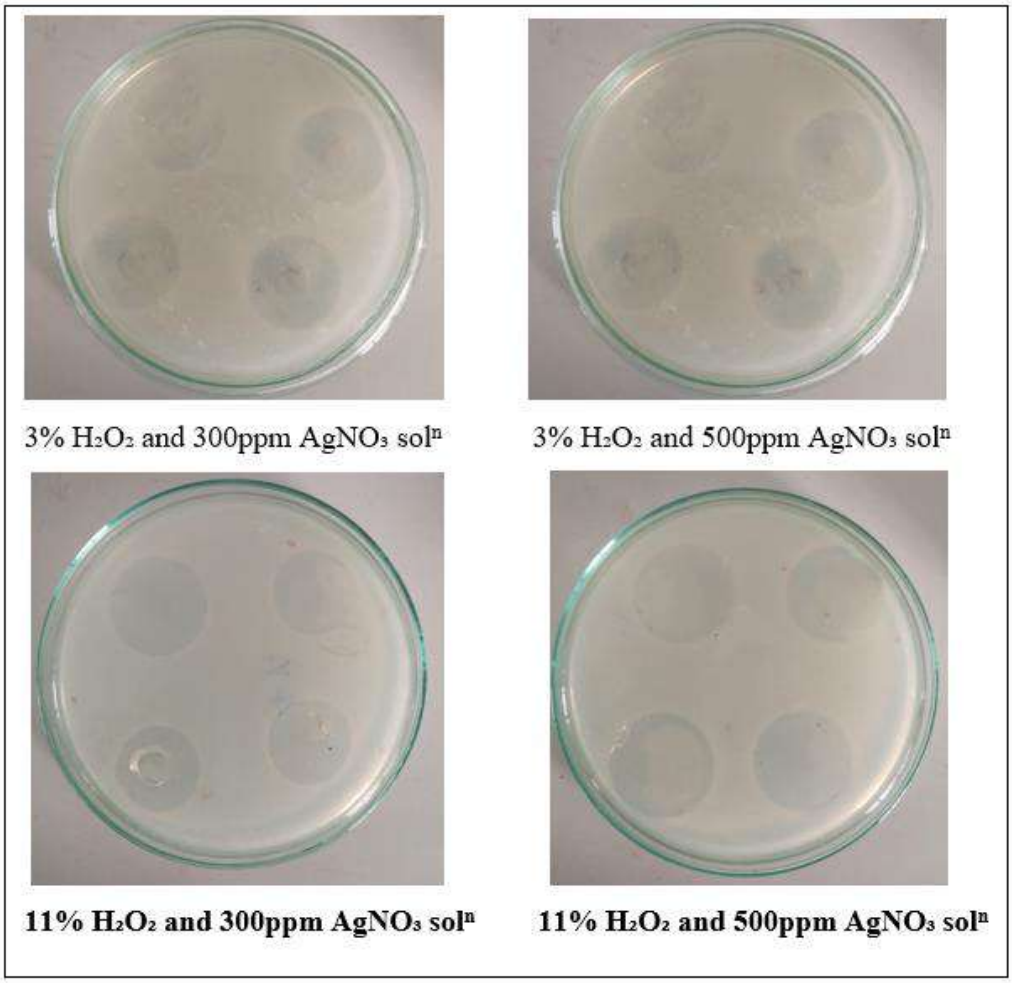

Figure. 5.1. Observation for Zone of Inhibition (ZOI) Preliminary Trial Batches.

Combining hydrogen peroxide with silver nitrate significantly boosts antimicrobial efficacy, with the most pronounced effects observed at higher concentrations of both agents. This combination outperforms standard commercial disinfectants,

suggesting its potential as a superior alternative for microbial control.

5.2. Formulation Development and optimisation Hydrogen Peroxide and Silver Nitrate-Based Disinfection Solution

The experimental batch formulations of silver nano colloidal hydrogen peroxide solutions were prepared using varying concentrations of hydrogen peroxide (3–11% w/w), aromatic amide stabilizer (1.0–2.5 mg/kg), and silver nitrate (100–500 ppm), with Water for Injection added up to 100%. The mixtures were processed at speeds of 20 to 40 RPM for 4 hours at a controlled temperature of 25–30°C. The final pH of the solutions ranged from 4.5 to 4.6.

Table.5.3. Experimental Batch Formulations of Silver Nano Colloidal Hydrogen Peroxide Solution

Component	Batch A	Batch B	Batch C
Hydrogen Peroxide (w/w %)	11.00%	11.00%	11.00%
Aromatic Amide Stabilizer (mg/kg)	1.0 mg/kg	2.0 mg/kg	2.5 mg/kg
Silver Nitrate (ppm)	100 ppm	300 ppm	500 ppm
Water (q.s.)	Up to 100%	Up to 100%	Up to 100%
Mixing Speed	20 RPM	30 RPM	40 RPM
Mixing Time	4 hours	4 hours	4 hours
Temperature	25–30°C	25–30°C	25–30°C
pH (final solution)	4.5	4.6	4.6

5.2.1. Analytical Characterization of Formulated Disinfection Solution Batches Containing Hydrogen Peroxide and Silver Nitrate.

The analytical characterization of the silver nano colloidal hydrogen peroxide batches showed consistent H_2O_2 assay value 11% (v/v). Silver nanoparticle sizes varied, with Batch B exhibiting the smallest range (150–190 nm) and Batch A the largest (250–350 nm). Zeta potential ranged from –18 mV to –32 mV, indicating varying degrees of colloidal stability. PDI values (0.428–0.728) suggested moderate particle size distribution. Residual stabilizer levels matched the input concentrations (1–2.5 mg/kg), and dry residue remained below 2.0 mg/kg. Visually, Batches B and C were clear and colorless, while Batch A showed slight opalescence.

Table .5.4. Analytical Characterization of Disinfection Solution Batches Containing Hydrogen Peroxide and Silver Nitrate.

Parameter	Batch A	Batch B	Batch C
H_2O_2 Assay (% v/v)	90.20%	91.60%	93.70%
Silver Nanoparticle Size (nm)	250 – 350 nm	150 – 190 nm	120 – 140 nm
Zeta Potential (mV)	-13.0 mV	-12.7 mV	-12.1 mV
Polydispersity Index (PDI)	0.728	0.478	0.428
Residual Stabilizer (mg/kg)	1	2	2.5
Dry Residue (mg/kg)	<1.5	<2.0	<2.0
Visual Appearance	Clear, Colorless	Clear, Colorless	Slight Opalescence

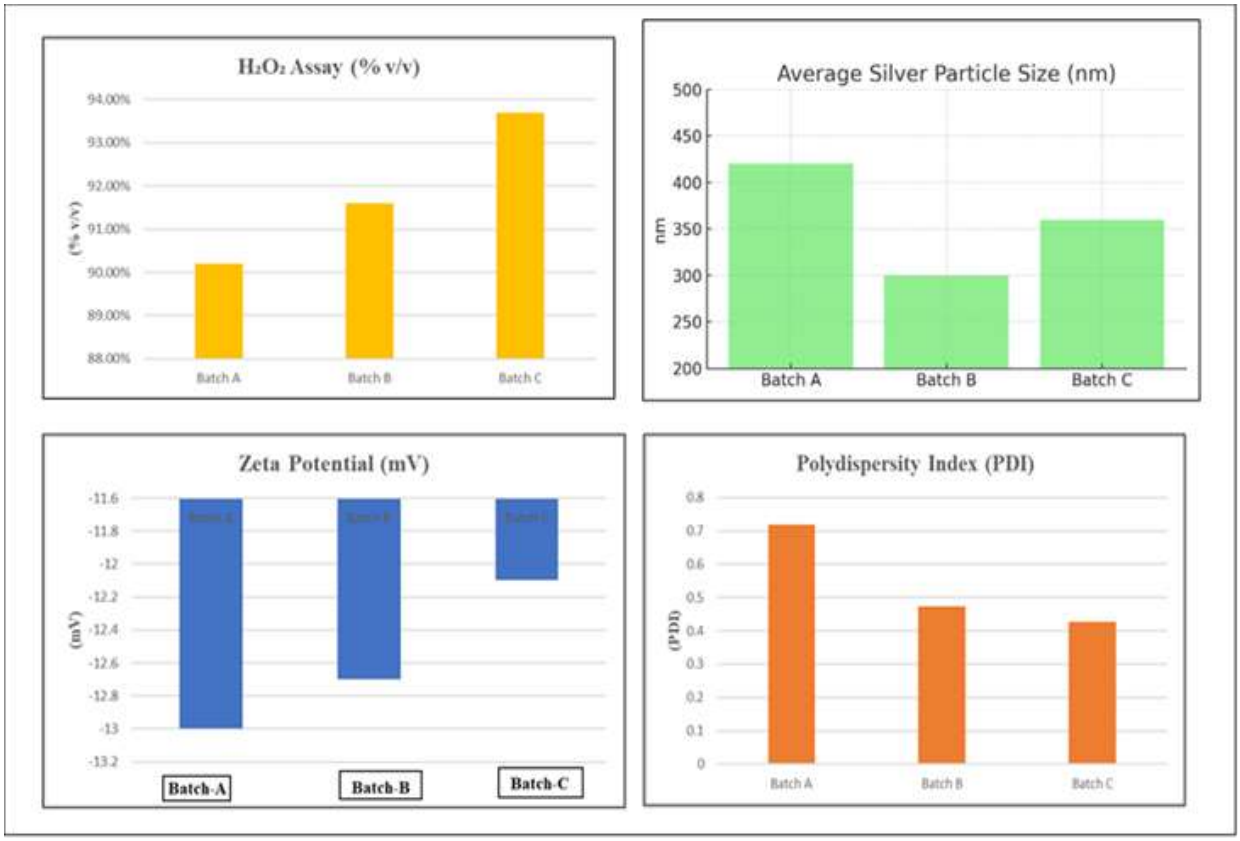

Figure.5.2. Analytical Characterization of Disinfection Solution Batches Containing Hydrogen Peroxide and Silver Nitrate.

5.2.2. Optimized Final Batch of Hydrogen Peroxide and Silver Nitrate-Based Disinfection Solution.

Among the three batches, **Batch C** demonstrated the best balance of stability and performance metrics. It maintained an excellent H_2O_2 assay (93.70%), ideal silver particle size (120–140 nm), and favourable zeta potential (−12.7 mV), indicating strong colloidal repulsion and dispersion. The PDI of 0.428. suggested a moderately narrow particle size distribution, appropriate for sustained stability. The dry residue was kept below 2.0 mg/kg across all batches, making them suitable for pharmaceutical and agricultural use. While Batch A showed slightly broader particle size distribution and mild opalescence, it still met acceptable limits for application. **Batch C** was selected as the optimized composition for further development and industrial scale-up.

Figure.5.3 Optimized Final Batch-C Of Disinfection Solution Batches Containing Hydrogen Peroxide and Silver Nitrate.

Table 5.5. Optimized Final Batch-C of Disinfection Solution Batches Containing Hydrogen Peroxide and Silver Nitrate.

Parameter	Batch C
H_2O_2 Assay (% v/v)	93.70%
Silver Nanoparticle Size (nm)	120 – 140 nm
Zeta Potential (mV)	-12.1 mV
Polydispersity Index (PDI)	0.428
Residual Stabilizer (mg/kg)	2.5
Dry Residue (mg/kg)	< 2.0
Visual Appearance	Slight Opalescence

5.2.3. Characterization of Optimized Batch of Disinfection Solution Batch Containing Hydrogen Peroxide and Silver Nitrate.

A. Clarity:

The optimized batch of nano silver hydrogen peroxide solution exhibited excellent clarity, as evident from the image, with no visible turbidity, particulate matter, or sedimentation. The solution appeared transparent and colorless, indicating successful dispersion of silver nanoparticles and absence of any instability or aggregation. This high clarity confirms the physical stability and homogeneity of the formulation, making it suitable for aerial disinfection applications where uniform particle dispersion and visual transparency are critical for performance and quality assurance.

Figure.5.4. Clarity of Optimized Batch of Disinfection Solution Containing Hydrogen Peroxide and Silver Nitrate.

B. Stability study of Disinfection Solution Batches Containing Hydrogen Peroxide and Silver Nitrate.

Stability study of Nano Silver Hydrogen Peroxide Solution Showes in table. 8.8.

Table.5.6. Stability study of Disinfection Solution Batches Containing Hydrogen Peroxide and Silver Nitrate.

Stability Study Report of Disinfection Solution Batches Containing Hydrogen Peroxide and Silver Nitrate.

Formulation Batch	month	Appearance	pH	% Assay	Particle size in nm	Zeta potential mV	Polydispersity Index (PI)
Specification		Clear transparent liquid	1-3	90-94%	120 -140	-13.0 to -12.1	0.728-0.428
Batch-C	0	Clear transparent liquid	2.53	90.58	138.0	-13.0	0.50
	1	Clear transparent liquid	2.61	92.50	129.4	-12.7	0.450
	2	Clear transparent liquid	2.75	93.70	126.8	-12.1	0.428

5.4. In-vitro Performance Evaluation/Efficacy study:

In vitro performance assessment and effectiveness investigation of fogging-based disinfection employing silver nanoparticles and hydrogen peroxide. This method is commonly employed in controlled environments, such as laboratories or cleanrooms, to assess the antimicrobial effectiveness of disinfectants. In the setup shown, a fogging device disperses a fine mist of hydrogen peroxide and silver nanoparticles into the air, ensuring widespread coverage of all surfaces and airborne spaces. The fog acts as a disinfectant, targeting and eliminating microbial contaminants. The study typically involves microbial sampling before and after fogging to determine the reduction in microbial load and evaluate the disinfectant's efficacy. The combined action of hydrogen peroxide, known for its oxidative damage to microbial cells, and silver nanoparticles, which disrupt microbial membranes and interfere with cellular functions, provides a synergistic antimicrobial effect. This type of study is essential for validating the use of such disinfection methods in healthcare, pharmaceutical, and laboratory settings where sterility and contamination control are critical.

Figure. 5.5. Fogging of Hydrogen peroxide and silver nitrate

Based disinfectant solution.

5.4.1. Calculate Total volume of area (Room):

- 1^{st} area+ 2^{nd} area = Total area
- 1^{st} area volume = length(3m) ×width (2.5m) ×Hight (3.30m) =24.75m^3
- 2^{nd} area volume= length (1.6m) ×width (1.4m) ×Hight (3.30m) =7.392m^3

1^{st} area+ 2^{nd} area = Total area

=24.75m^3+ 7.392m^3

=32.142m^3

- Total volume of area (Room)= 32.142m^3

5.4.2. Before fogging of disinfectant solution take counting of microbes, bacteria and fungi on specific agar medium:

Before the application of the disinfectant solution via fogging, microbial load assessments were conducted on various agar media including General (R2A) Agar, MacConkey Agar, Blood Agar, and Sabouroud Dextrose-Chloramphenicol Agar to quantify bacteria and fungi present in the environment. The initial microbial counts served as a baseline to evaluate the effectiveness of fumigation. Post-fumigation microbial sampling was performed at intervals of 15, 30, and 60 minutes. Across all agar types, there was a significant increase in colony-forming units (CFU/m^3) with time after fumigation. On General (R2A) Agar, CFU/m^3 counts increased from an average of 20 at 15 minutes to 66 at 60 minutes. MacConkey Agar showed a rise from 3.2 to 9 CFU/m^3, Blood Agar from 18.8 to 53 CFU/m^3, and Sabouroud Dextrose-Chloramphenicol Agar (used to isolate fungi) from 6.8 to 12 CFU/m^3. These results indicate the persistence and gradual

reappearance of airborne microbes after fumigation, emphasizing the need for time-based monitoring and potentially repeated disinfection for effective microbial control.

Table.5.7. Before fogging of disinfectant solution take counting of microbes, bacteria and fungi on specific agar medium

Agar		General(R2A)			MacConkey Agar			Blood Agar			Sabouroud Dextrose-Chloramphenicol Agar		
Time (min)		15	30	60	15	30	60	15	30	60	15	30	60
After Fumigation microbial counting (CFU/m^3)	1	25	45	62	2	4	6	28	40	52	7	8	10
	2	18	30	70	4	5	8	15	39	50	7	11	13
	3	30	15	50	1	3	5	21	45	57	5	7	10
	4	20	40	80	4	9	14	16	38	51	6	7	11
	5	7	40	68	5	12	12	14	41	55	9	13	16
Total CFU/m^3		20	34	66	3.2	6.6	9	18.8	40.6	53	6.8	9.2	12

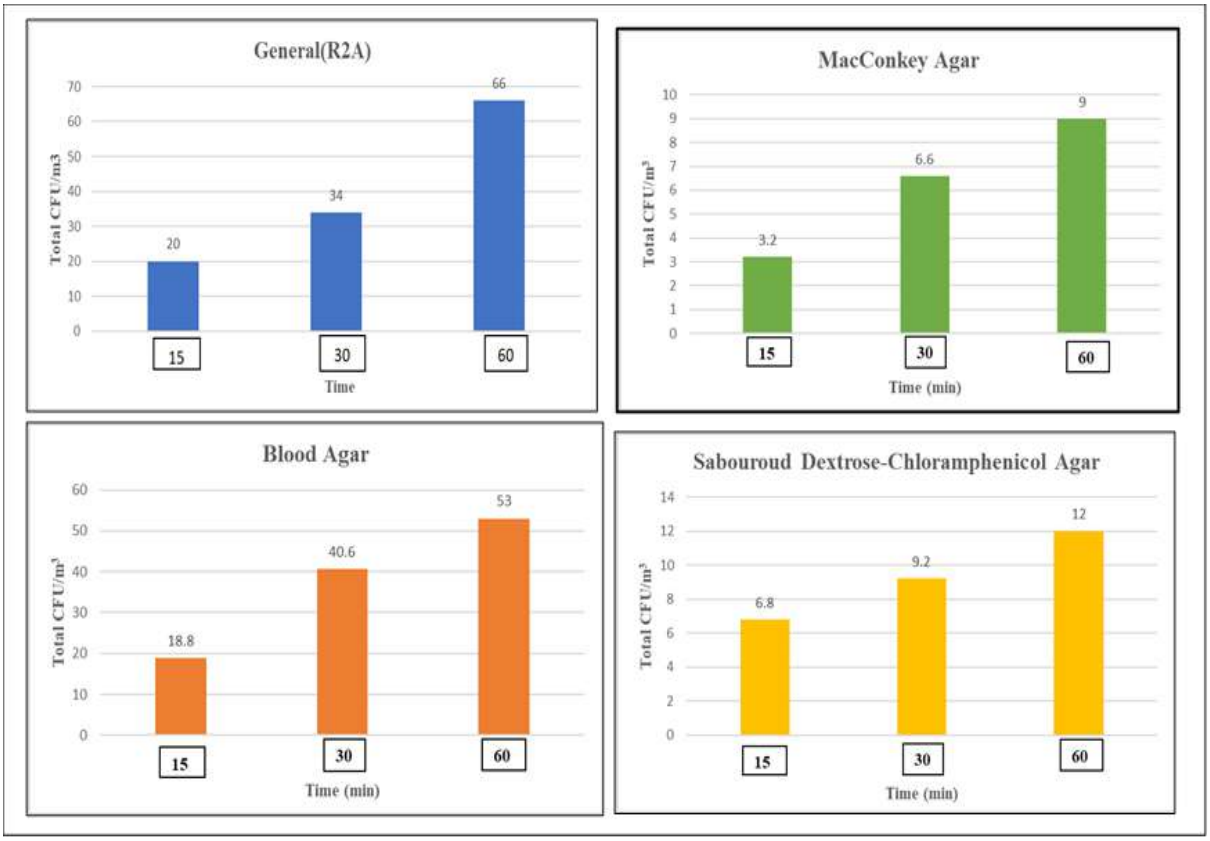

Figure.5.6 Graphical representation Before fogging of disinfectant solution take counting of microbes, bacteria and fungi on specific agar medium

5.4.3. After one month fogging of Hydrogen peroxide 11% and silver nitrate 300 ppm (Batch-B) disinfectant solution.

Following fogging with a disinfectant solution consisting of 11% hydrogen peroxide and 300 ppm silver nitrate, microbial counts were assessed at intervals of 15, 30, and 60 minutes using four different agar media: General (R2A) Agar, MacConkey Agar, Blood Agar, and Sabouraud Dextrose-Chloramphenicol Agar. The results showed low microbial concentrations, indicating a significant reduction in airborne microorganisms. On General (R2A) Agar, average CFU/m³ values

were 0.4 at 15 minutes, 0.8 at 30 minutes, and 2.6 at 60 minutes. MacConkey Agar showed a slight increase over time, with values of 1, 1.8, and 3 CFU/m³. Blood Agar recorded a gradual rise from 5 to 9.4 CFU/m³, while fungal growth observed on Sabouraud Dextrose-Chloramphenicol Agar increased from 2.8 to 6.8 CFU/m³ over the 60-minute period. These findings demonstrate that the disinfectant was effective in reducing the microbial load immediately after application, although a gradual resurgence of bacteria and fungi was observed with time. The overall microbial recovery remained low, suggesting that the disinfectant at this concentration provides considerable but time-limited control of airborne contaminants.

Table.5.8. One month After fogging of Hydrogen peroxide 11% and silver nitrate 300 (Batch-B) ppm disinfectant solution observe the bacteria.

Agar		General(R2A)			MacConkey Agar			Blood Agar			Sabouroud Dextrose-Chloramphenicol Agar		
Time (min)		15	30	60	15	30	60	15	30	60	15	30	60
After Fumigation microbial counting (CFU/m³)	1	1	0	1	0	1	1	8	10	12	2	6	8
	2	0	1	0	1	0	2	5	9	10	3	4	6
	3	0	1	3	0	1	2	2	5	7	4	5	7
	4	1	2	5	2	4	5	6	8	10	2	3	5
	5	0	0	4	2	3	4	4	6	8	3	5	8
Total CFU/m³		0.4	0.8	2.6	1	1.8	3	5	7.6	9.4	2.8	4.6	6.8

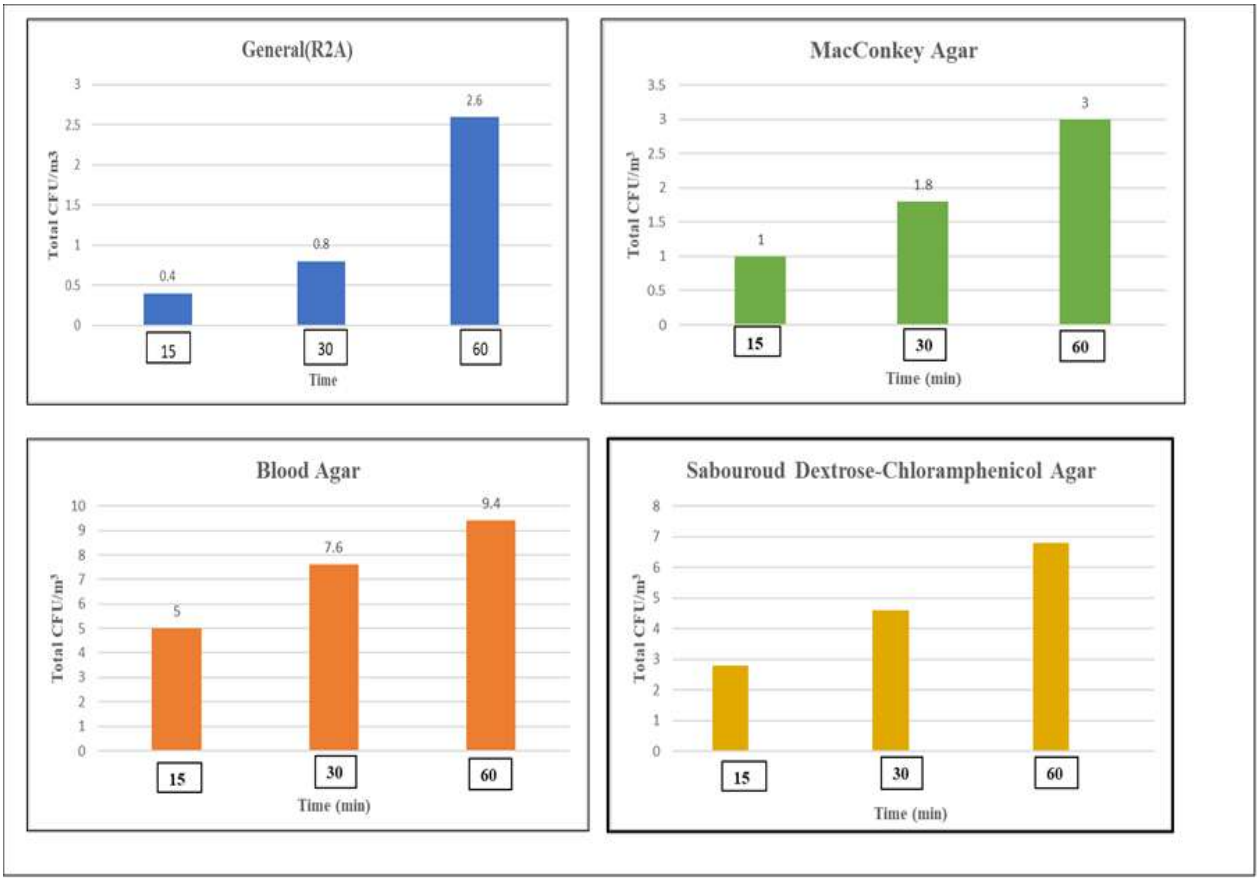

Figure.5.7. Graphical representation One month after fogging of Hydrogen peroxide 11% and silver nitrate 300 ppm (Batch-B) disinfectant solution take counting of microbes, bacteria and fungi on specific agar medium

5.4.4. After one month fogging of Hydrogen peroxide 11% and silver nitrate 500 ppm (Batch-C) disinfectant solution

After fogging with a disinfectant solution composed of 11% hydrogen peroxide and 500 ppm silver nitrate, a significant reduction in airborne microbial load was observed across all tested media. Microbial sampling was conducted at 15, 30, and 60 minutes post-fumigation using General (R2A) Agar, MacConkey Agar, Blood Agar, and Sabouraud Dextrose-Chloramphenicol Agar. The results revealed notably low colony-forming unit (CFU/m³) counts during the monitoring period. On General (R2A) Agar, CFU/m³ values remained minimal, increasing only slightly from 0.3 at 15 minutes to 2.1 at 60 minutes. MacConkey Agar also showed very low microbial presence, with CFU/m³ rising from 0.9 to just 2.6 over the same interval. Blood Agar recorded a gradual increase from 4.6 to 9.0 CFU/m³, while Sabouraud Dextrose-Chloramphenicol Agar, used to detect fungi, showed a moderate rise from 2.1 to 6.1 CFU/m³. These findings indicate the disinfectant was highly effective in reducing microbial contamination immediately after fogging, with only a gradual and limited reappearance of microorganisms observed over the course of one hour.

Table.5.9. One month After fogging of Hydrogen peroxide 11% and silver nitrate 500 ppm(Batch-C) disinfectant solution observe the bacteria.

Agar		General(R2A)			MacConkey Agar			Blood Agar			Sabouroud Dextrose-Chloramphenicol Agar		
Time (min)		15	30	60	15	30	60	15	30	60	15	30	60
After Fumigation microbial counting (CFU/m^3)	1	1	0	1	0	1	1	8	10	12	2	6	8
	2	0	1	0	1	0	2	5	9	10	3	4	6
	3	0	1	3	0	1	2	2	5	7	4	5	7
	4	1	2	5	2	4	5	6	8	10	2	3	5
	5	0	0	4	2	3	4	4	6	8	3	5	8
Total CFU/m^3		0.3	0.7	2.1	0.9	1.4	2.6	4.6	7.1	9.0	2.1	4.0	6.1

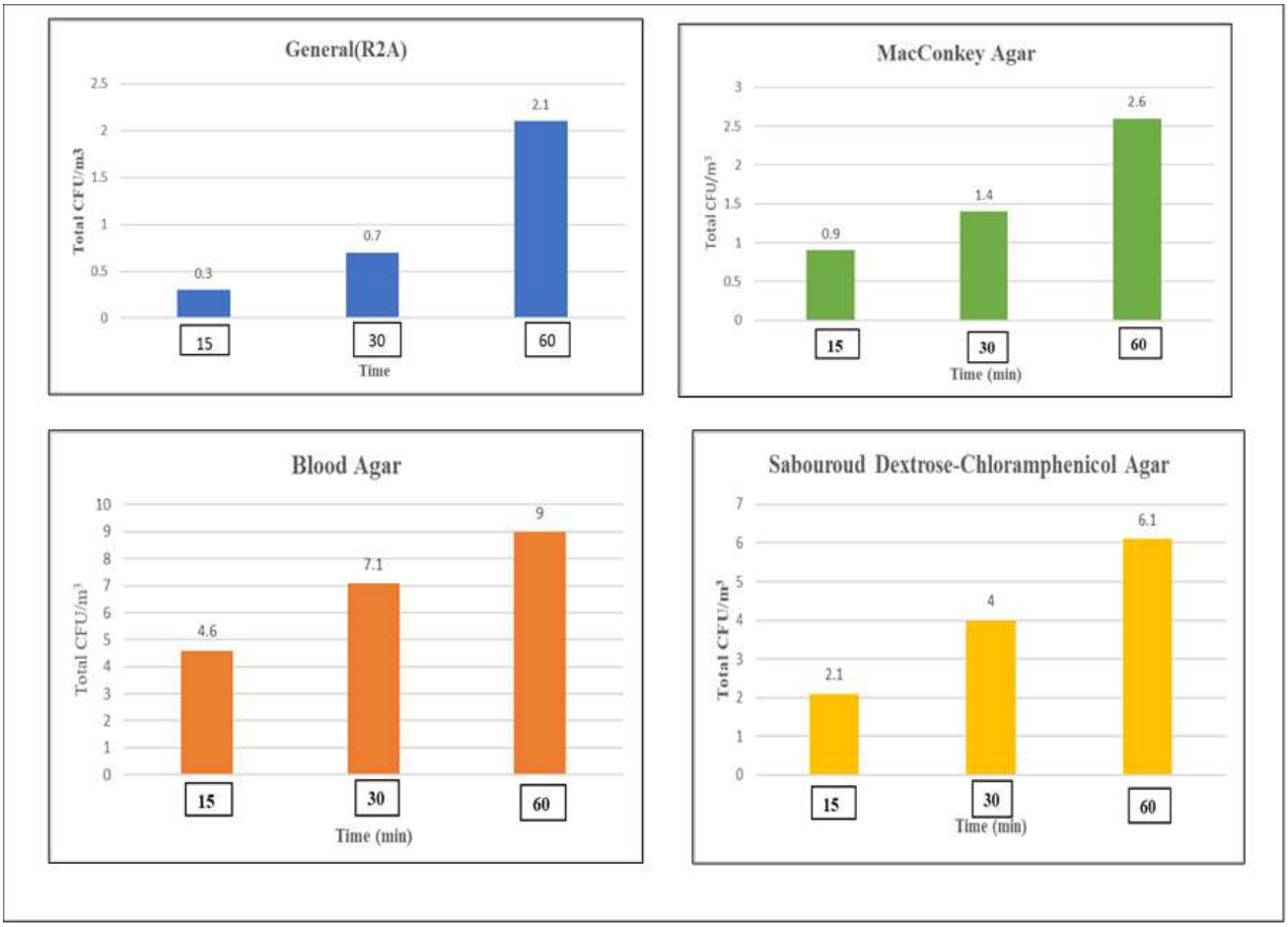

Figure.5.8. Graphical representation One month after fogging of Hydrogen peroxide 11% and silver nitrate 500 ppm (Batch-C) disinfectant solution take counting of microbes, bacteria and fungi on specific agar medium

6.0. CONCLUSION

This study aimed to the optimization arial disinfection solution. Batch C was identified as the optimal formulation due to its high H_2O_2 content (93.70%), ideal silver nanoparticle size (120–140 nm), and stable colloidal properties (zeta potential −12.1 mV, PDI 0.428). With low dry residue (<2.0 mg/kg) and slight opalescence, it meets the quality standards for pharmaceutical and agricultural use, making it suitable for further development and scale-up combination of hydrogen peroxide (11%) with silver nitrate at concentrations of 300 ppm and 500 ppm presents a highly effective and promising disinfectant solution, offering broad-spectrum antimicrobial efficacy, improved physical stability, and consistent homogeneity. The formulations, particularly Batch-B and Batch-C, demonstrated favourable particle size, uniform distribution (as indicated by low PDI), and stable zeta potential values, suggesting reliable performance and colloidal stability. The acidic pH, ranging from 3.24 to 3.55, contributes further to antimicrobial activity, while container compatibility studies confirm that amber glass provides optimal long-term stability. Despite the observed turbidity and precipitation, these characteristics are inherent to silver-based nanoparticle formulations and do not compromise the solution's uniformity or performance. While the potential for dermal and inhalation irritation exists more pronounced in the 500 ppm

concentration these risks can be effectively mitigated through standard safety practices, such as using protective equipment and proper ventilation during handling. Overall, the optimized formulations combine potent disinfection capability with favourable physicochemical properties, making them suitable candidates for applications such as fogging and surface decontamination in healthcare and high-risk environments. An in-vitro fogging efficacy study further assessed the antimicrobial performance of these formulations. Air sampling on various agar media conducted before and after fogging at 15, 30, and 60 minute intervals revealed a significant initial microbial load. The 300 ppm formulation effectively reduced microbial presence, although some regrowth was observed over time. In contrast, the 500 ppm formulation sustained lower microbial counts throughout all time points, indicating superior and prolonged antimicrobial activity. Overall, the findings confirm that fogging with a combination of hydrogen peroxide and silver nanoparticles is an effective strategy for short-term microbial reduction in controlled environments.

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