EFFICIENCY OF Ef- Chlor Bio- Tab/ Effi- Sept/ Effi- Kleen 1.67gm & 4gm WATER PURIFICATION AND SURFACE DISINFECTANT TABLET AND GRANULES AND ITS ANTIMICROBIAL ACTIVITY AGAINST BROAD SPECTRUM MICROORGANISMS IN POULTRY

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Abstract

The efficiency of Ef- Chlor Bio- Tab / Effi- Sept/ Effi- Kleen 1.67gm & 4gm water purification and surface disinfectant tablets and granules containing sodium dichloroisocyanurate (NaDCC) as major components was tested against microbes collected in sterile vials from a poultry farm and identified using a microscope. The tests were carried out in accordance with US EPA guidelines, with physical parameters such as pH, odour, hardness, and so on of the water being tested, as well as chemical testing of the feed and litter to determine the toxicity present. In biological parameters, microbial cultures were prepared in broth and are serially diluted up to 10 dilutions, then microbes were cultured at 35-37°C using the pour plate method in sets of three, one is referred as control, and the CFU (Cell Forming Unit) of each plate was counted. To determine the kill time of tablets and granules, a log reduction procedure (up to 10 logs) was used by treating each plate except control with the desired tablet dilution. CFUs of each plate were counted again at various time intervals (5 minutes, 10 minutes, 15 minutes, and 20 minutes and so on respectively). Water, feed, and litter were also tested before and after EF- Chlor treatment.

***Keywords*:** Ef- Chlor, NaDCC, US EPA guidelines, microbial cultures, pour plate method, CFU (Cell Forming Unit), log reduction and kill time.

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Introduction

Maintaining the quality of poultry drinking water is an important nutritional consideration because birds consume twice as much water as that of feed. Water quality is determined by a variety of factors, including microbial levels, pH, mineral content, hardness, and organic matter load, all of which must be within acceptable limits to ensure water quality [1]. Chicken feed may contain water sources contaminated with urine and feces, as well as litter, which exposes birds to pathogens both directly and indirectly [2]. The most frequent cause of bacterial illnesses in chickens is an opportunistic organism present in the surroundings of poultry [3], because of this; sanitation is a crucial component of hatchery design and administration. This involves following excellent hygiene procedures, which are necessary for the highest hatching rate and chick quality [4]. The employment of the "all-in/all-out" approach (i.e., all birds within an establishment must be of the same age group) is one of the most crucial requirements for sanitary and hygienic facilities, with the constraint that the same type or species of bird be employed in each organization. [5].

The sodium salt of chlorinated hydroxy-triazine, sodium dichloroisocyanurate (NaDCC), is another approach for producing free, readily available chlorine. Formaldehyde was widely used as a conventional chemical disinfectant in the disinfection of eggs prior to NaDCC, which can leave toxic residues and impair hatchability, chick quality, and chick growth performance (Oliveira et al., 2020) [6], This also has a negative impact on embryos and is hazardous to the health of farm and hatchery workers (Zeweil et al., 2015, Kusstatscher et al., 2017). As a result, alternative products that can provide satisfactory sanitation without reducing embryo incubation efficiency or harming the professionals involved in the process are required.

Temperature is important in the development of chicks; thus, the chick room should be kept at 22°C with a relative humidity of about 25% and proper ventilation. Similarly, the hatcher room must have a temperature of approximately 24°C and a relative humidity of approximately 50% [7].

Steps Involved in the Sanitization Process

- **STEP- 1:**

  Sanitization at the Entrance of Premise:

  At the entry, vehicles, tyres, storage racks/ crate and bird cages should be cleaned with 1000ppm Ef-Chlor tablets. After shoes were sanitized at 200ppm, it was recommended to wrap them with polyethylene shoe covers (for dilutions, please see the directions for use table).

Fig. 1 (a); SANITATION OF SHOES AT ENTRANCE (200ppm)

Fig.1 (b); SANITATION OF VEHICLES AT ENTRANCE (1000ppm)

- **STEP- 2:**

  Disinfection:

  Disinfecting office floor by mopping it with 1000ppm and contact surface such as door, handles, table, chair, etc must be cleaned at 4000ppm with Ef-Chlor Bio- Tab/ Effi- Sept/ Effi- Kleen 1.67gm and 4gm tablet.

  The hatchery's floor, walls, ceiling as well as hatchery equipment were cleaned with 4000 ppm of Ef- Chlor Bio- Tab/ Effi- Sept/ Effi- Kleen 1.67gm and 4gm tablet. Feed for broilers are also disinfected using 15gm granules of Ef-Chlor (for dilutions, please refer to the directions for use table).

Fig. 2; DISINFECTION OF HATCHERY EQUIPMENTS (4000ppm)

- **STEP- 3:**

  Sterilization of Egg Surface in Incubation Room:

  Fumigation is used to sterilize egg surfaces in two ways. Firstly, use of 50gm $KMnO_4$ in 100ml formalin at 37°C.

  Secondly, a small amount of para-formaldehyde was heated at room temperature before being used as a fumigator.

  The use of para-formaldehyde has been found to be hazardous, so it is advised that eggs were fumigated at 1000ppm using Ef-

Chlor Bio- Tab/ Effi- Sept/ Effi- Kleen 1.67 gm tablets. Before fumigating eggs, the fumigator room must be sterilized by mopping the floor at 4000ppm and at 10,000ppm once in a week for better result (for dilutions, please refer to the directions for use table).

- **All of the above methods were carried out in a fumigator chamber for about 10 to 15 minutes, where these chemicals were placed alongside egg trays, and the fumes were spread uniformly with the help of a fan located above the chamber.**

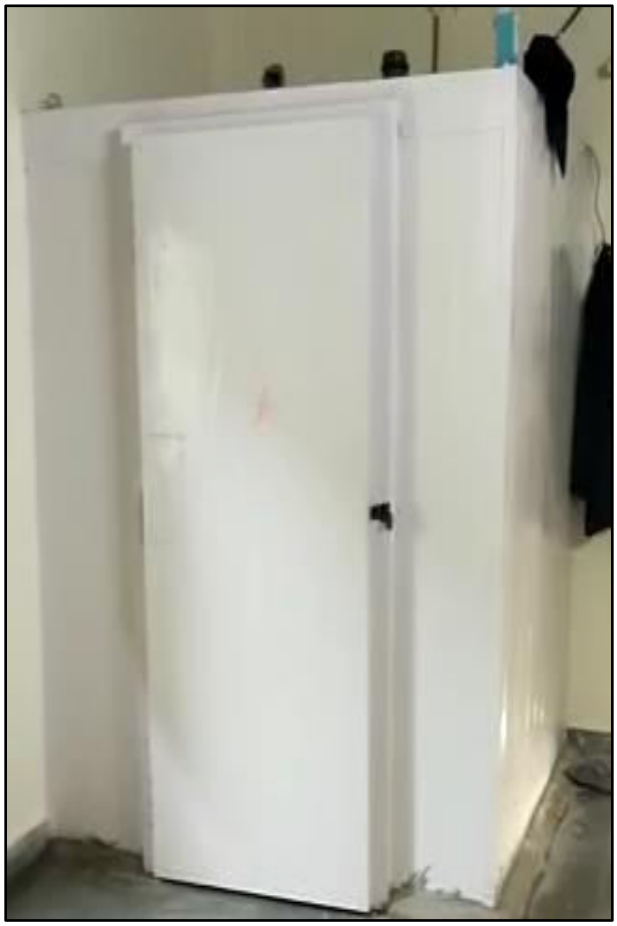


Fig. 3; FUMIGATOR CHAMBER

- **STEP- 4:**

Incubation of Eggs:

Following the fumigation process, the egg trays were placed in an incubation chamber where humidity and temperature were maintained at different stages of development, and the egg trays were tilted at an angle of 45º every hour.

Fig. 4; INCUBATOR CHAMBER

- During the first week, the eggs were incubated at 38°C.

- The temperature should be 35-36°C in the second week.
- The temperature should be between 30-32°C in the third week.
- The temperature is lowered to 26–28°C when the chicks hatch in the fourth week of development and is kept there throughout sales.
- **STEP-5:**

  Racks & Trays for Storage/ Transportation Management:

  It is important to disinfect the tray at 4000ppm before transferring hatched chicks to sheds (for dilutions, please refer to the guidelines for usage table).
- **STEP- 6:**

  Litter Management for Shed Preparation:

  Before moving chicks to the sheds for additional nourishment and development, it is necessary to disinfect the litter. This is done by simply combining Ef- Chlor granules with CaCO3 and the litter (for dilutions, please refer to the recommendations for usage table). To absorb moisture from excreta, a 2 inch thick layer of the mixture is applied throughout the shed.

  Daily ploughing of the litter is necessary for the comfort of the chicks.

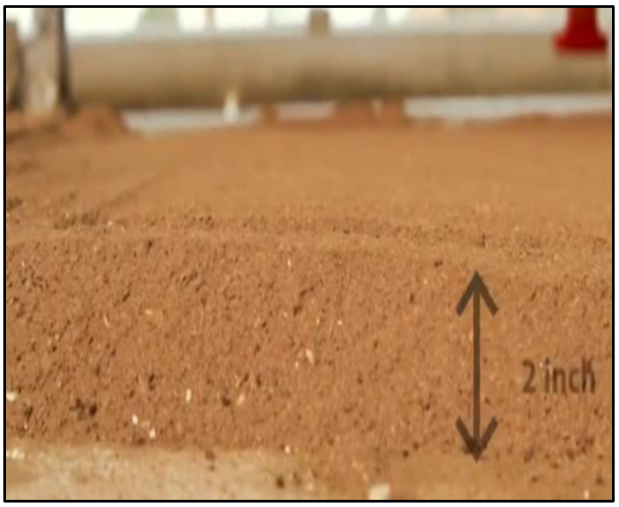


Fig. 5; LITTER MANAGEMENT

- **STEP- 7:**

  Transportation of Hatched Chicks to Shed:

  After four weeks of incubation, the chicks were transported in trays to the sheds for further development.
- **STEP- 8:**

  Feeding of Newly Hatched Chicks in Shed:
- **Nutrition:**

  Newly hatched chicks were fed with fodder high in protein containing maize and soybeans, which was mixed with some oil.
- **Drinking Water:**

  Shock chlorination is a technique for sterilizing pipe lines and flushing water tanks after cleaning in order to guarantee that the water tanks and pipelines are free of microorganisms like bacteria, fungi, and other similar things. This procedure is to be

carried out at 100 ppm of Ef- Chlor Bio- Tab/ Effi- Sept/ Effi- Kleen 1.67gm and 4gm solution [8]. Before shock chlorination, make sure there is no residual material in the pipelines or tank during the process.

After this process, one tablet of Ef-Chlor 1.67gm and 4gm tablet (2.0ppm) was dissolved in 500 liter tank and left for 30 minutes before using it to feed the chicks.

Fig. 6; DRINKING WATER TANK (2.0ppm)

- **Humidity:**

  In order to maintain humidity, a humidifier that is attached to a 500-liter tank in the shed is placed, and it periodically sprays water (2.0ppm) that contains Ef- Chlor Bio- Tab/ Effi- Sept/ Effi- Kleen 1.67gm and 4gm throughout the space and all chicks present.

- **Ventilation:**

  By installing exhaust fans in the poultry shed, it is possible to increase the ventilation along with maintaining the temperature up to a maximum of 28°C.

- **STEP- 9:**

  Transportation of Chicken in Cage:

  Prior to transportation, the cage and vehicle are thoroughly disinfected with a 1000ppm Ef- Chlor Bio- Tab/ Effi- Sept/ Effi- Kleen 1.67gm and 4gm solution (for dilutions, please see the directions for use table).

- **STEP-10:**

  Disinfection of Deceased Chicks & Hens:

  In order to disinfect dead chickens, the dead chicks & hen were then thoroughly cleaned, submerged in Ef- Chlor-treated water (4000ppm) for 30 minutes, and then buried deeply in the ground with Ef- Chlor granule (10- 20gm) sprinkled on it.

- **SHED-11:**

  Waste Management:

  The shed litter was taken out after around 10 days and sold to farmers to be utilized as manure in agricultural farms, where it is safe for agricultural use because it has been treated with Ef- Chlor.

Direction for Use of Ef-Chlor

Table 1; Direction for use of Ef- Chlor Bio- Tab/ Effi- Sept/ Effi- Kleen 1.67gm & 4gmTablets

S.NO.	Direction for use	Volume in ppm	Contact time	Number of Tablet Used for the dissolution of Ef- Chor Bio- Tab/ Effi- Sept/ Effi- Kleen 1.67gm and 4gm tablets	Number of Tablet Used for the dissolution of Ef- Chor Bio- Tab/ Effi- Sept/ Effi- Kleen 4gm tablets
1.	Sanitization at the Entrance of Premise and fumigation of eggs	200 ppm	2-3 minutes	1 tablets in 10 liters of water	2 tablets in 24 liters of water
2.	Disinfection hatchery equipment	1000ppm	30 minutes	1 tablet in 1 liters water	1 tablets in 2.0 liters of water
3.	Drinking Water	2.0ppm	30 minutes	2 tablets in 500 liters water	1 tablets in 500 liters of water
4.	Floor mopping	1000ppm	10 minutes	1 tablet in 1 liters water	1 tablets in 2.2 liters of water
		4000ppm	2 minutes	4 tablets in 1 liters water	1 tablets in 0.5 liters of water
		10,000ppm	Once a week	6 tablets in 1 liters water	4 tablets in 1 liters of water

Table 2; Direction for use of Ef- Chlor Granules

S.NO.	Direction for use	Volume in ppm	Contact time	Amount of Ef- Chor Granules Used for the Dissolution
1.	Litter management	N/A	2- 3 minutes	5kg Ef-Chlor granule in 500gm $CaCO_3$ mixed in 50kg litter
2.	Disinfection of deceased chicks & hens	4000ppm	30 minutes	6.7gm Ef- Chlor granules in 1 liters water

METHODS for ANALYSIS

1) **Collection of Samples**:

Samples from the hatchery (from the incubation room and fumigator room handle and floor), shed (from the walls and floor), office gate and handle, and other locations are collected using swab rods and stored in broth in vials before and after Ef- Chlor Bio- Tab/ Effi- Sept/ Effi- Kleen 1.67gm and 4gm tablet and granule treatment. Similarly, surface samples from eggs were collected and stored. The samples were taken from a poultry farm in Neelbad, Madhya Pradesh, 462044.

2) **Identification of Microbes:**

Rapid detection and identification of microorganisms is a difficult and important feature in industries ranging from manufacturing to medicine. Standard methodologies (for example, culture media and biochemical tests) are known to be time-consuming and labor- intensive.

Gram's staining method, which involved creating slides using both broth or Petri plate cultures and an inoculating loop in a sterile environment with laminar air flow, was used to identify bacteria. Similar to this, fungus under sterile conditions are stained with lacto phenol cotton blue stain and then viewed and identified using light microscope slides.

3) **Antimicrobial Activity of Ef- Chlor Bio- Tab / Effi- Sept/ Effi- Kleen 1.67gm & 4 gm Tablet**:

- Prepare three sets of Petri plates (one is considered as control) using the pour plate method under laminar air flow with nutrient agar media that was autoclaved at 121°C for 15 minutes, and then let it solidified.
- Bacterial culture should be serially diluted 1:9 up to 10 times.
- Pour a small amount of the broth's pure culture into the control petri plate (designate it as the control) and spread it out evenly. To do the same, pour the culture from the sixth and tenth dilution and name it log 6 and log 10 respectively.
- The petri plates should be incubated for 24-48 hours at 35-37 °C.
- Following incubation, colonies generated on petri plates needed to be counted using a colony counter.
- Compare the microbial population in an Ef-Chlor- treated plate to an untreated plate (i.e. control).
- **For dilutions, please refer to the directions for use table.**
- Record raw data as CFU/plate to determine surviving organisms. To calculate CFU/mL of test suspension, each replicate dilution was multiplied by the dilution factor. After that, the CFU count should be converted to

log_{10} scale (up to 10 log scale).

- **Both CFU and antimicrobial activity must be measured before and after Ef-Chlor treatment.**

4) **Monitoring of feeds**:

To avoid contamination and the growth of microbes, chicken feed must be properly dried.

5) **Washing**:

The premises, bird crate, and dwelling area must all be thoroughly cleaned with Ef-Chlor Bio- Tab/ Effi- Sept/ Effi- Kleen 1.67gm and 4gm tablet and granule of concentration up to 4000ppm, then left undisturbed so that these areas can dry completely before the chicken were brought again.

6) **Decontamination of Water System**:

To clean the water system and fragmented tubes fill them with a water treated with Ef-Chlor Bio- Tab/ Effi- Sept/ Effi- Kleen 1.67gm and 4gm tablet and let to soak for 24 hours.

- **All of the methods described above were used to reduce or eliminate microbial populations that pose a threat to the flocks' health.**

RESULT

1. Identification of Microbes:

1.1 Bacterial Identification:

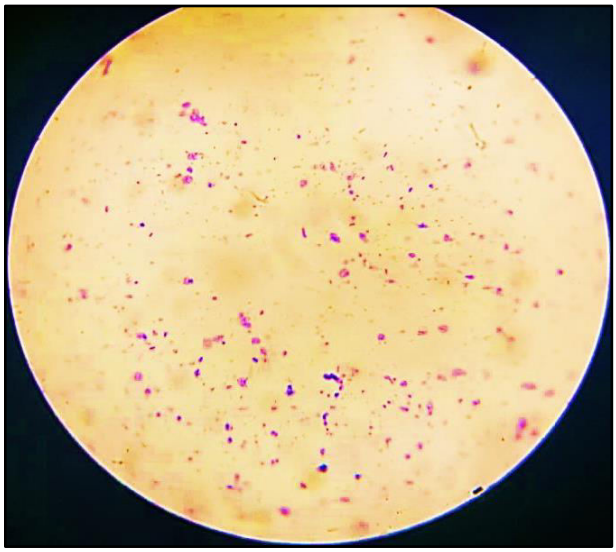
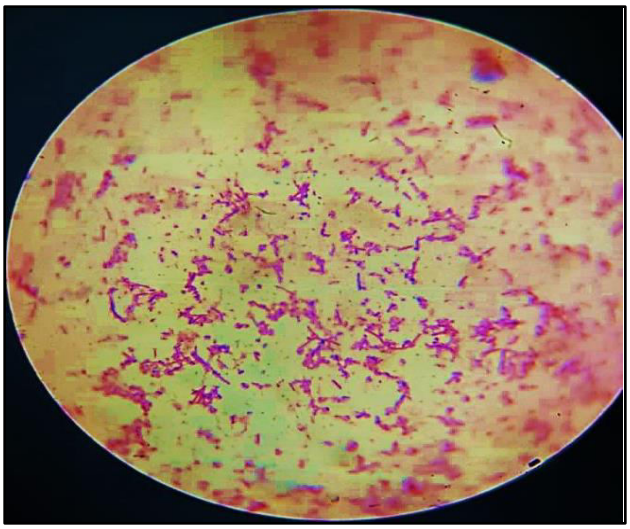


Fig. 7; SLIDE 1; SHED 7 FEEDING TRAY

Microbes identified:

- *Klebsiella pneumoniae*
- *Bacillus*



- *Staphylococcus aureus*

Fig. 8; SLIDE 2; SHED 7 DRINKER TRAY

Microbes identified:

- *Cocci*

- *Bacilli*
- *Staphylococcus aureus*

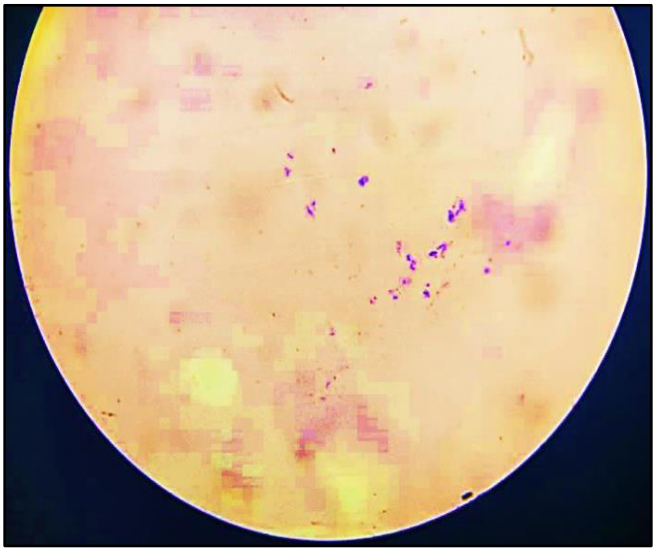


Fig. 9; SLIDE 3; SHED 7 WALLS

Microbes identified:

- *Klebsiella pneumoniae*

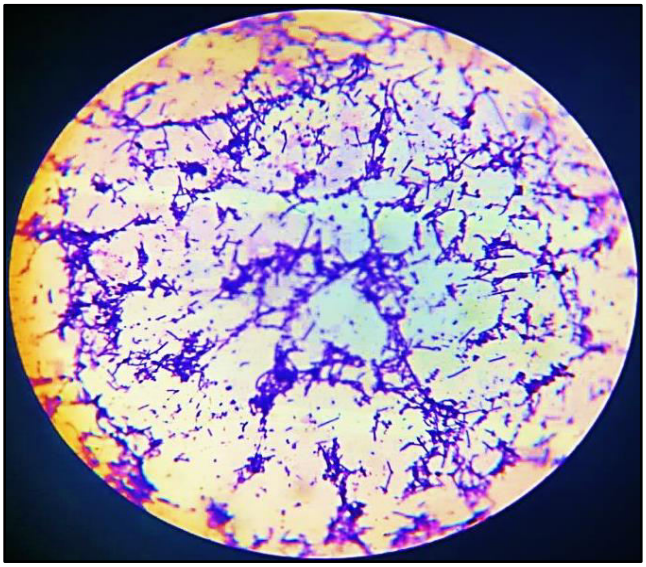


Fig. 10; SLIDE 4; SHED 7 FLOOR

Microbes identified:

- *Staphylococcus aureus*

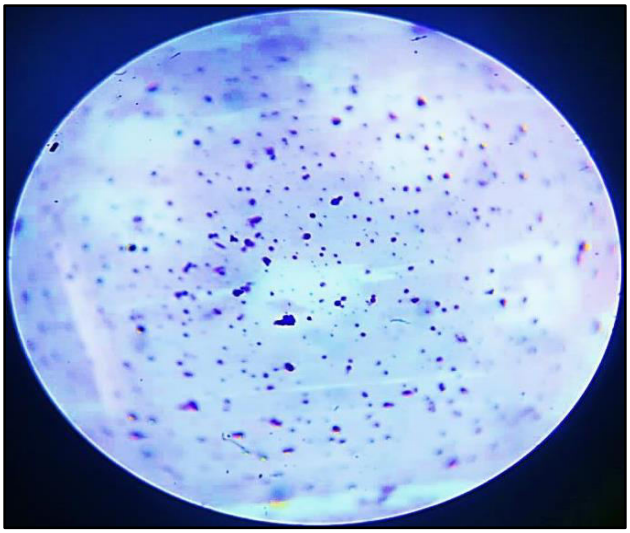


Fig. 11; SLIDE 5; EGG ROOM GATE HANDLE

Microbes identified:

- *Diplococci*
- *Cocci*
- *Clostridium botulinum*
- *Corynebacetrium diphtheriae*

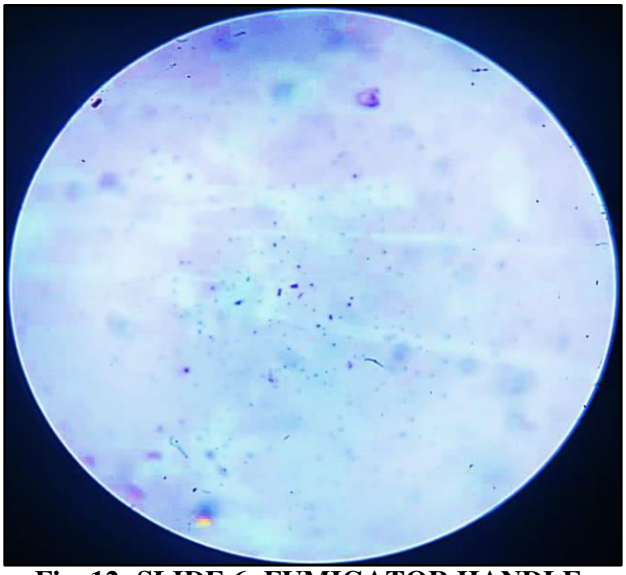


Fig. 12; SLIDE 6; FUMIGATOR HANDLE

Microbes identified:

- *Diplococci*
- *Cocci*

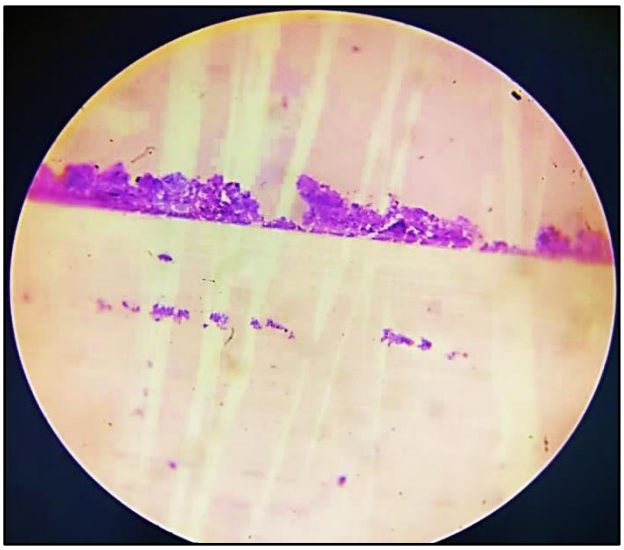


Fig. 13; SLIDE 7; FUMIGATOR FLOOR

Microbes identified:

- *Diplococcus*
- *Coccus*
- *Streptococcus pyogene*

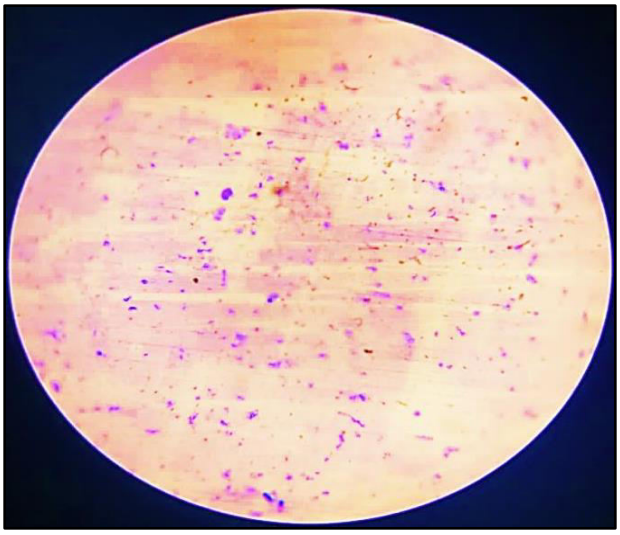


Fig. 14; SLIDE 8; INCUBATOR ROOM DOOR

Microbes identified:

- *Bacilli*
- *Corynebacetrium diphtheriae*
- *Vibrio cholerae*

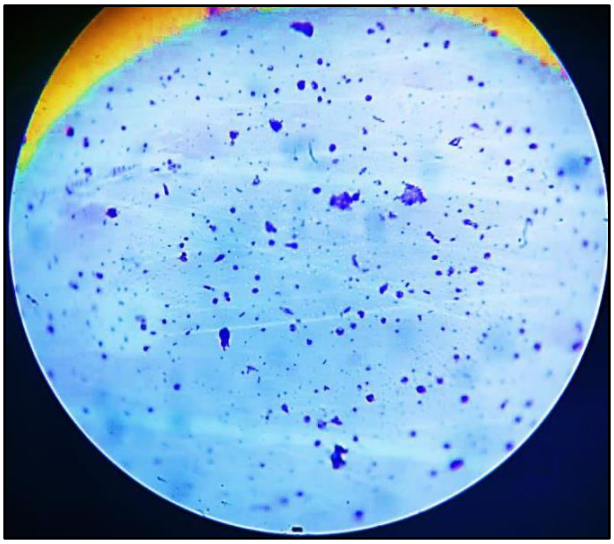


Fig. 15; SLIDE 9; INCUBATOR ROOM HANDLE

Microbes identified:

- *Staphylococcus aureus*
- *Streptococcus pneumoniae*
- *Cocci*
- *Corynebacterium diphtheria*

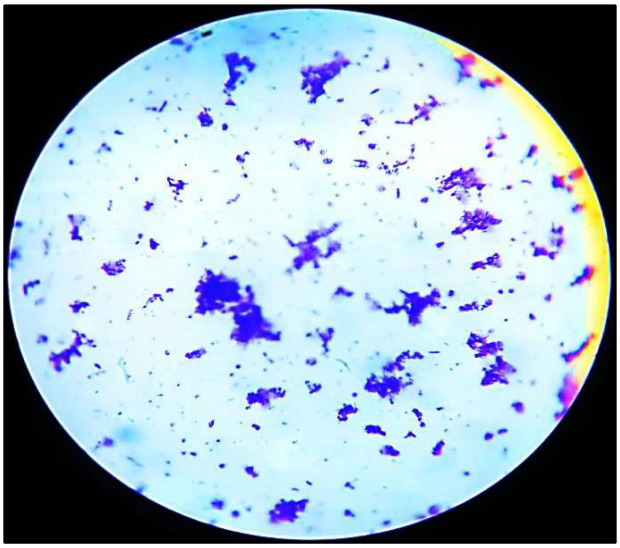


Fig. 16; SLIDE 10; MAIN GATE

Microbes identified:

- *Staphylopcoccus aureus*
- *Coccus*

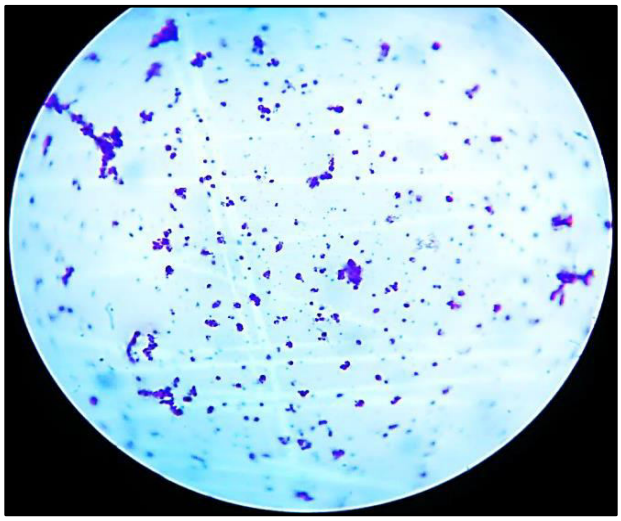


Fig. 17; SLIDE 11; OFFICE GATE

Microbes identified:

- *Staphylococcus aureus*
- *Bacillus*

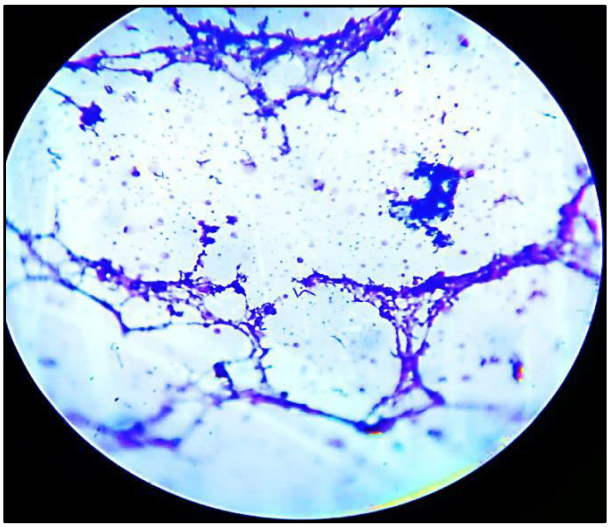


Fig. 18; SLIDE 12; HATCHERY TRAY

Microbes identified:

- *Staphylococcus aureus*

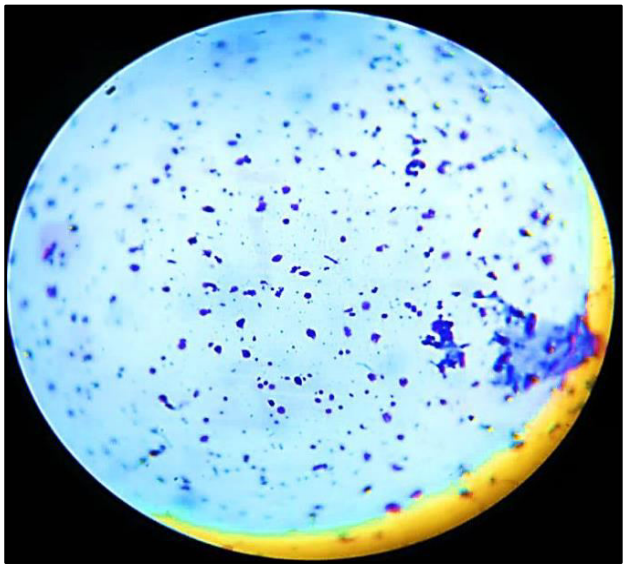


Fig. 19; SLIDE 13; HATCHERY FLOOR

Microbes identified:

- *Staphylococcus aureus*
- *Corynebacterium diphtheria*
- *Coccus*
- *Vibrio cholera*

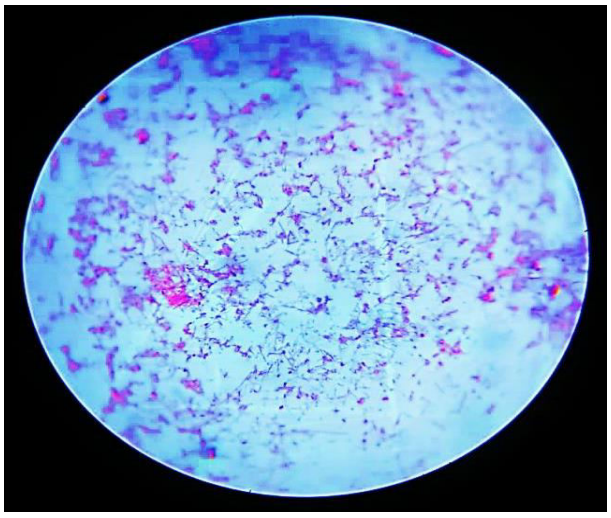


Fig. 20; SLIDE 14; SHED 2 (HEN) FLOOR

Microbes identified:

- *Corynebacterium diphtheria*
- *Bacillus substilis*

- *Staphylococcus aureus*
- *Vibrio cholera*

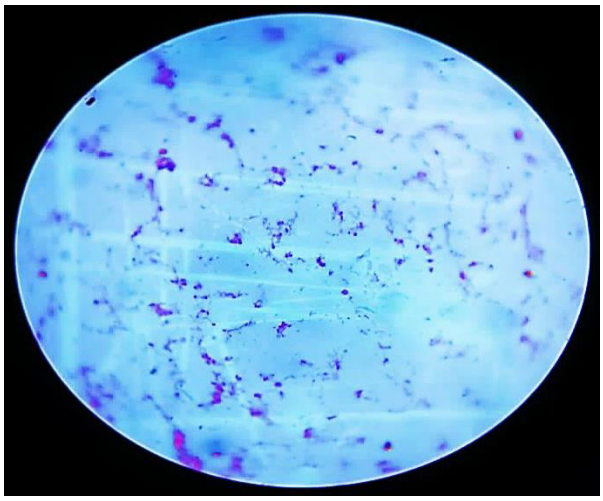


Fig. 21; SLIDE 15; SHED 2 (HEN) WALLS

Microbes identified:

- *Vibrio cholerae*
- *Corynebacterium diphtheria*
- *Staphylococcus aureus*

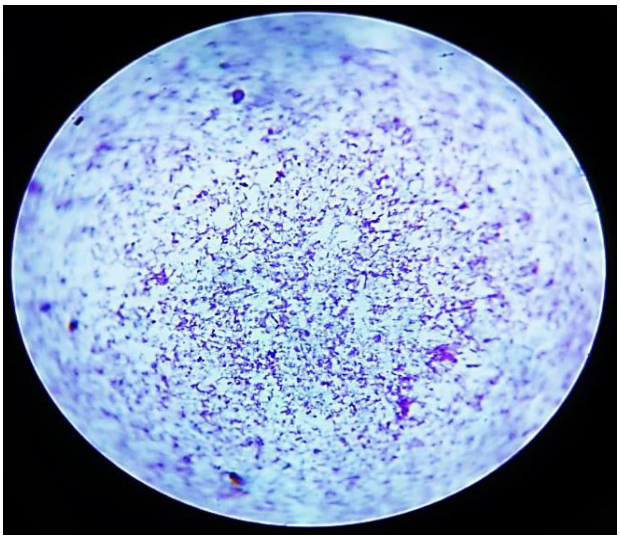


Fig. 22; SLIDE 16; UNTREATED LITTER

Microbes identified:

- *Vibrio cholera*
- *Corynebacterium diphtheria*
- *Staphylococcus aureus*
- *Streptococcus pyogene*

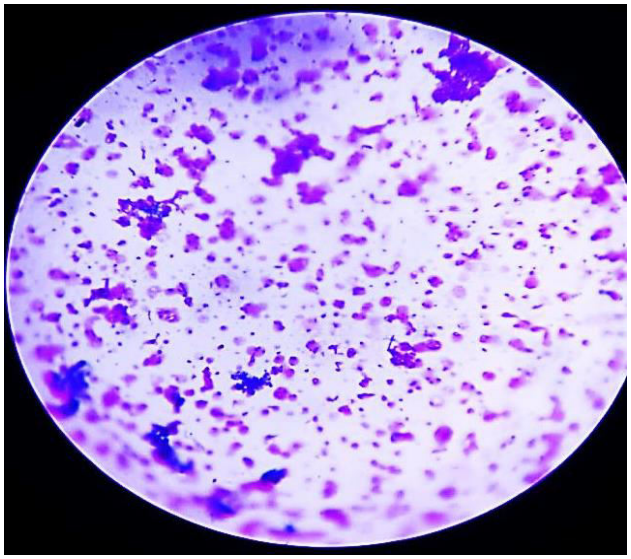


Fig. 23; SLIDE 17; EGG SURFACE

Microbes identified:

- *Staphylococcus aureus*
- *Streptococcus pneumoniae*
- *Coccus*
- *Clostridium tetani*

1.2 Fungal Identification:

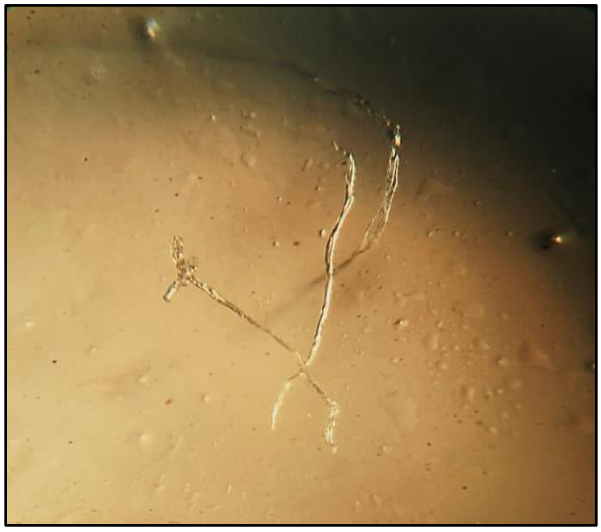


Fig. 24; SLIDE 1; FUMIGATOR ROOM HANDLE

Microbes identified:

- *Rhizopus*

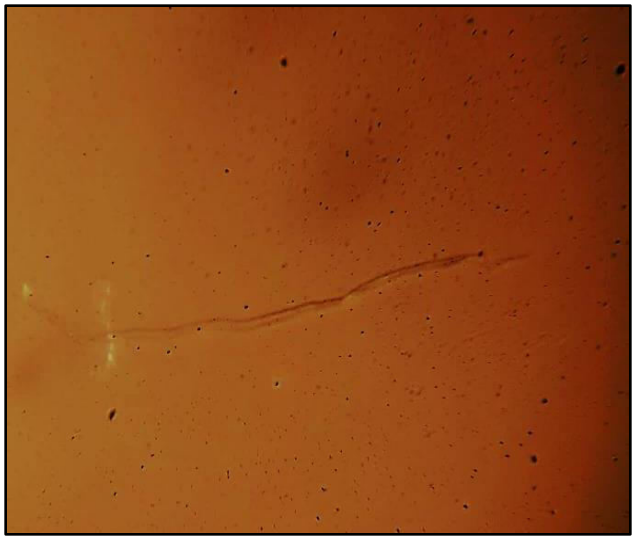


Fig. 25; SLIDE 2; SHED- 7 FEEDING TRAY

Microbes identified:

- *Rhizopus*

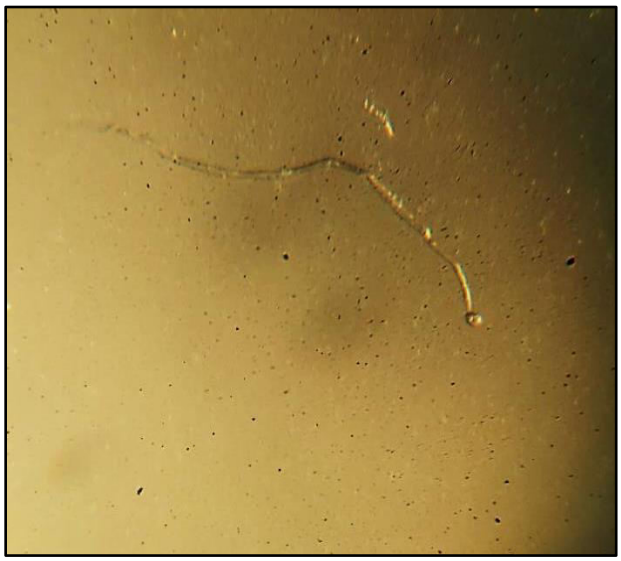


Fig. 26; SLIDE 3; FUMIGATOR FLOOR

Microbes identified:

- *Nigrospora*

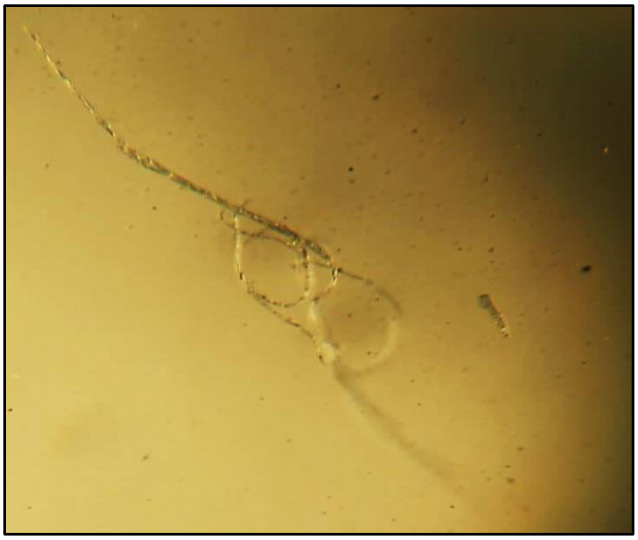


Fig. 27; SLIDE 4; SHED- 7 WALLS

Microbes identified:

- *Fusarium*

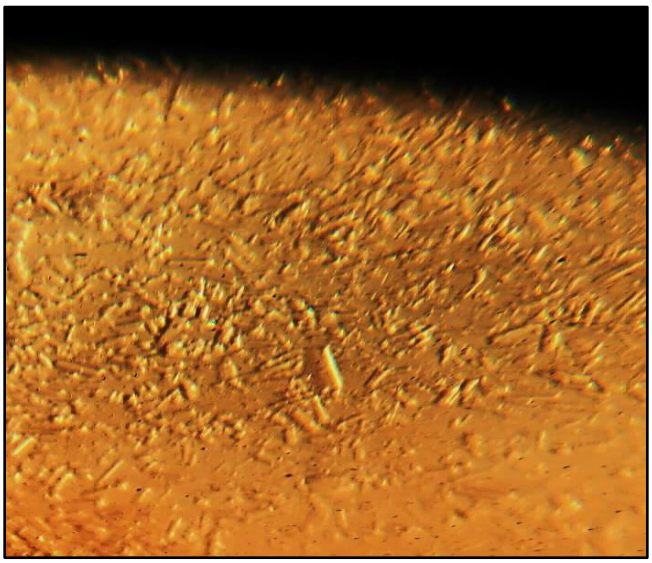


Fig. 28; SLIDE 5; SHED- 2 (HEN) WALLS

Microbes identified:

- *Fusarium*

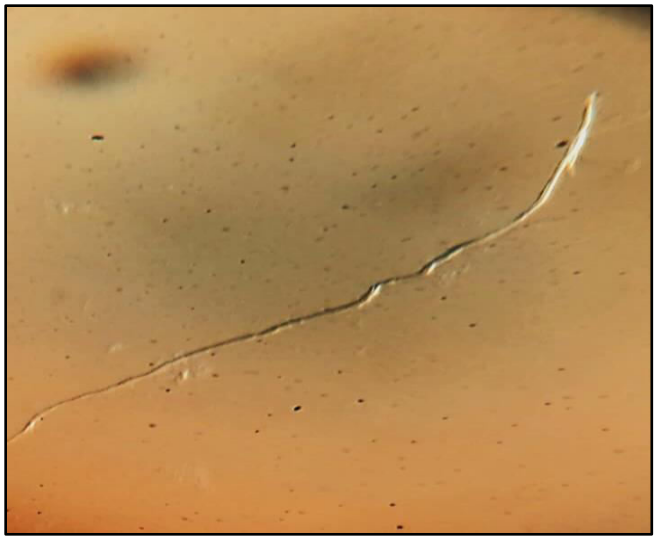


Fig. 29; SLIDE 6; EGG SURFACE

Microbes identified:

- *Fusarium*

Fig. 30; SLIDE 7; DRINKER TRAY

Microbes identified:

- *Fusarium*

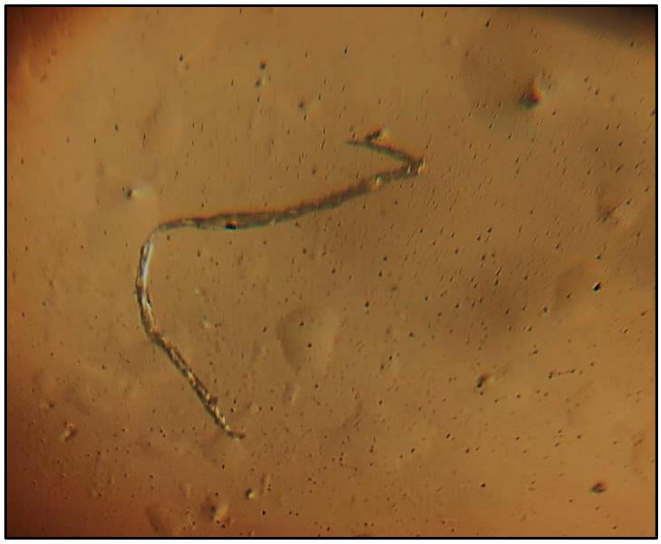


Fig. 31; SLIDE 8; EGG ROOM GATE HANDLE

Microbes identified:

- *Fusarium*

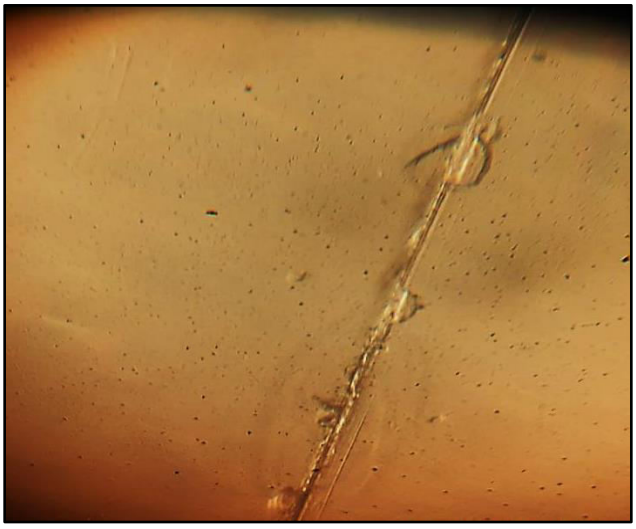


Fig. 32; SLIDE 9; HATCHERY FLOOR

Microbes identified:

- *Nigrospora*

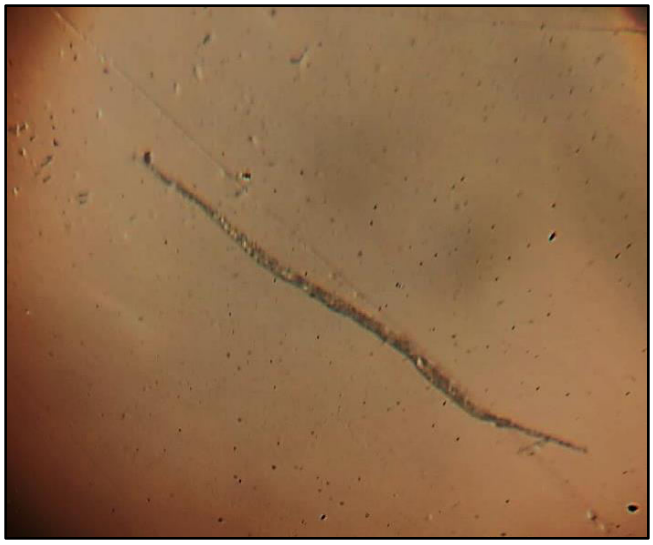


Fig. 33; SLIDE 10; HATCHERY TRAY

Microbes identified:

- *Fusarium*

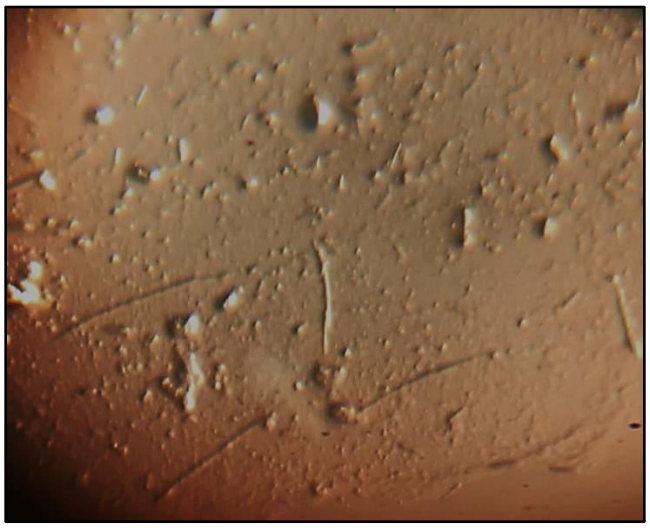


Fig. 34; SLIDE 11; INCUBATOR ROOM DOOR

Microbes identified:

- *Mucor*

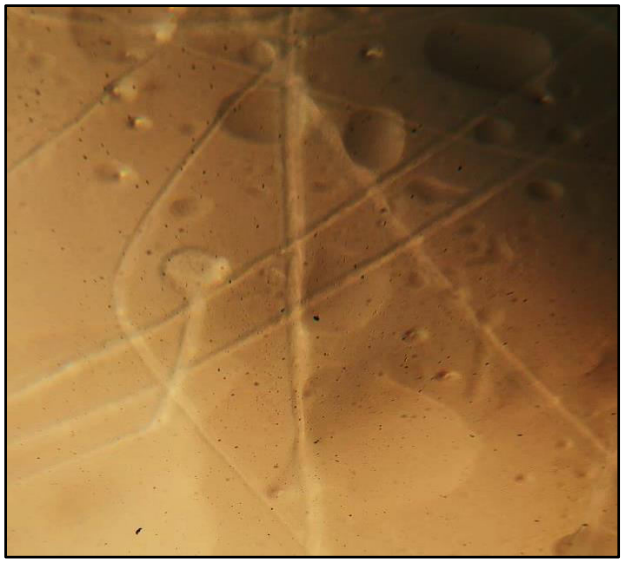


Fig. 35; SLIDE 12; INCUBATOR ROOM GATE HANDLE

Microbes identified:

- *Mucor*

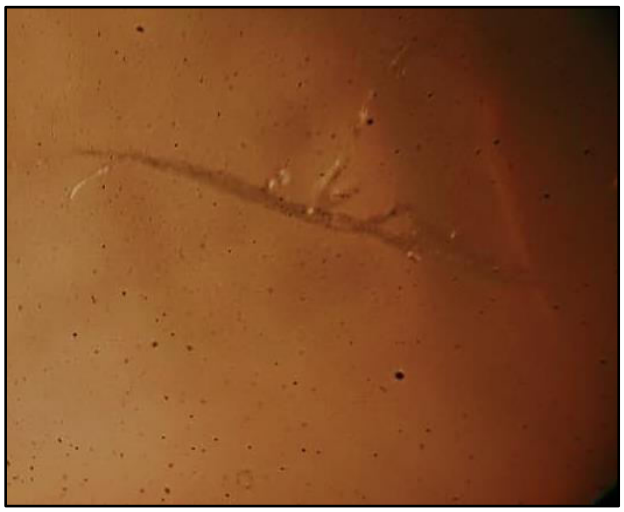


Fig. 36; SLIDE 13; OFFICE GATE

Microbes identified:

- *Rhizopus*

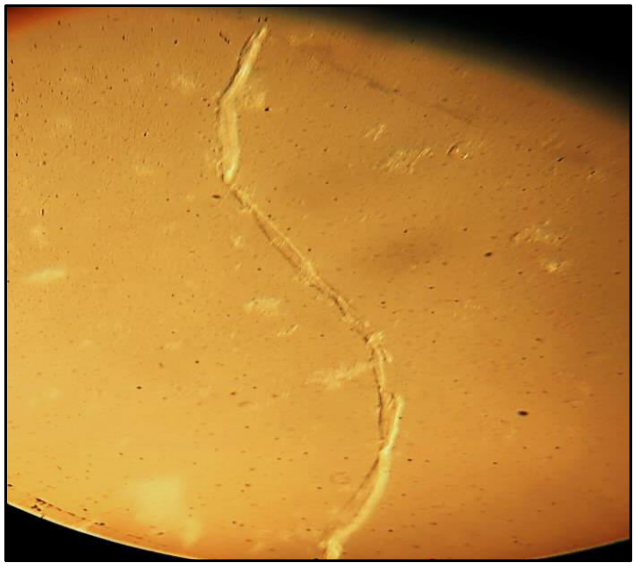


Fig. 37; SLIDE 14; MAIN GATE

Microbes identified:

- *Fusarium*

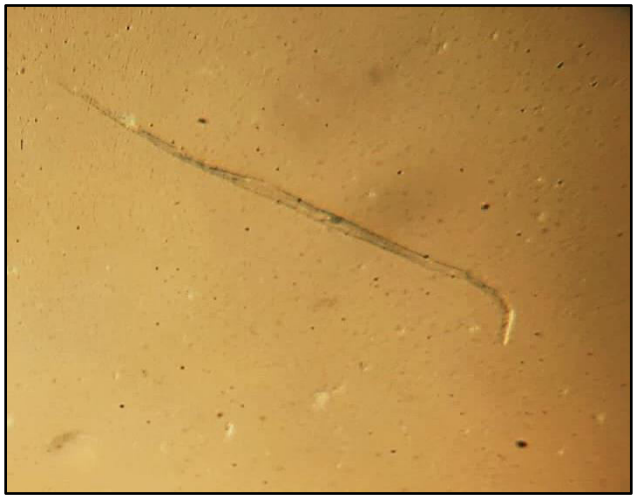


Fig. 38; SLIDE 15; LITTER

Microbes identified:

- *Fusarium*

2. Antimicrobial Activity of Ef- Chlor Bio- Tab/ Effi- Sept/ Effi- Kleen 1.67gm:

Except for the control sample, each sample was treated with Ef- Chlor Bio- Tab/ Effi- Sept/ Effi- Kleen 1.67gm and 4gm at a different ppm. After 24 hours of incubation, each sample's CFU were counted using a colony counter, and the results were recorded.

2.1 Against Bacteria:

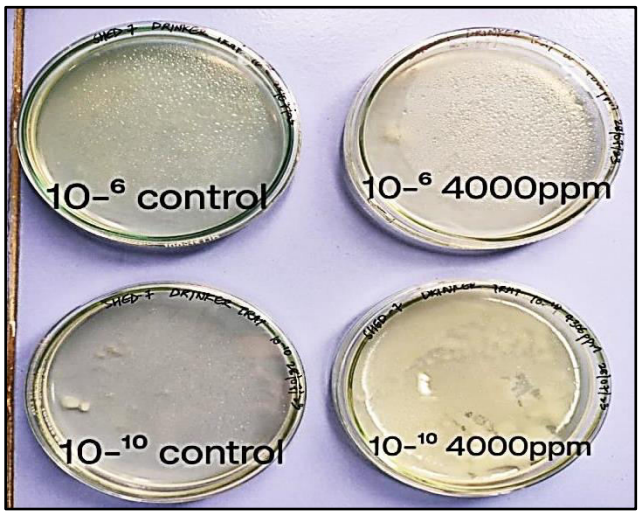


Fig. 39; SHED- 7 DRIKER TRAY

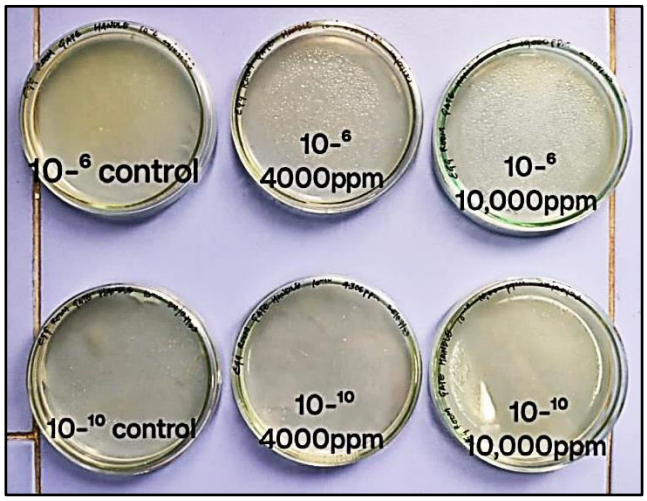


Fig. 40; EGG ROOM GATE HANDLE FLOOR

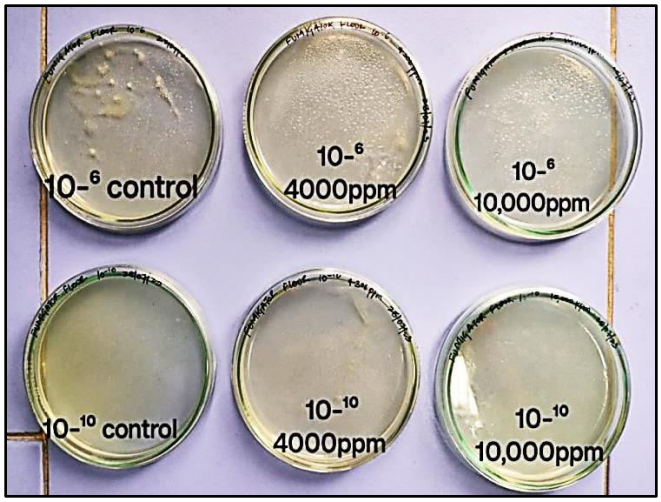


Fig. 41; FUMIGATOR FLOOR

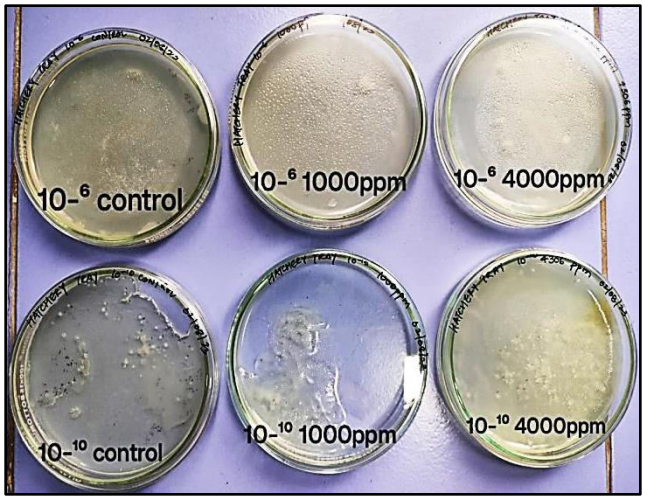


Fig. 44; HATCHERY TRAY

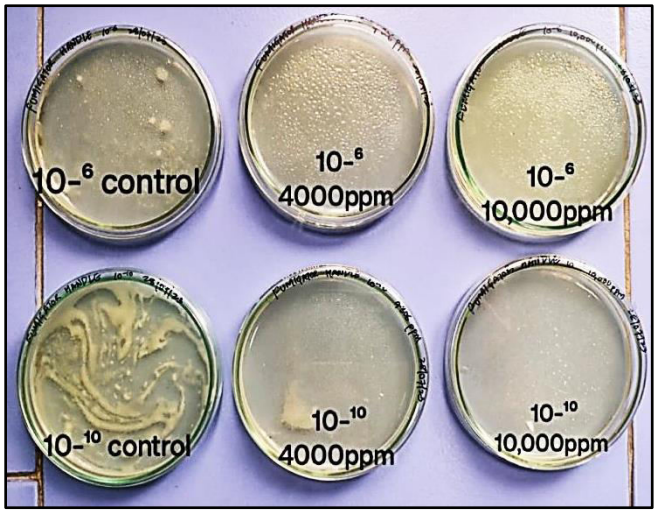


Fig. 42; FUMIGATOR HANDLE

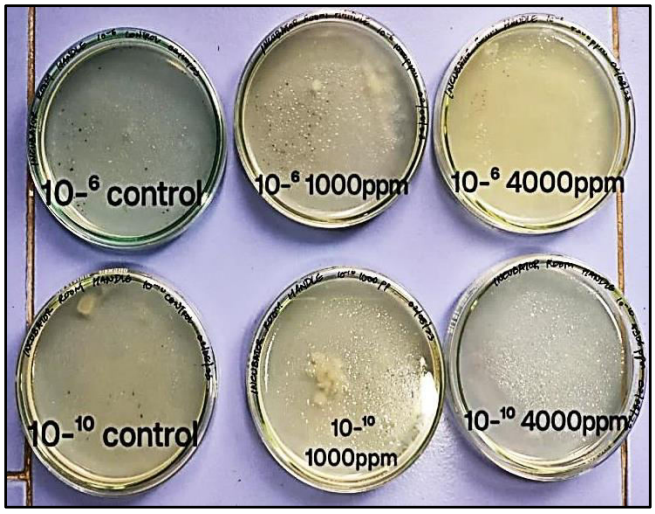


Fig. 45; INCUBATOOR ROOM HANDLE

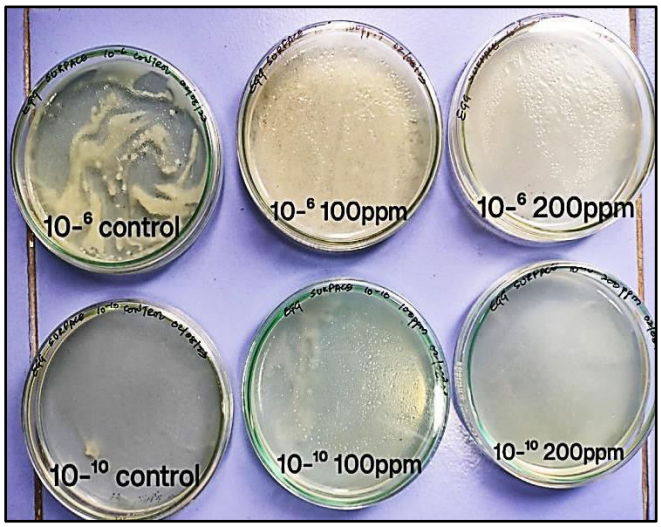


Fig. 43; EGG SURFACE

Fig. 46; SHED- 7 WALLS

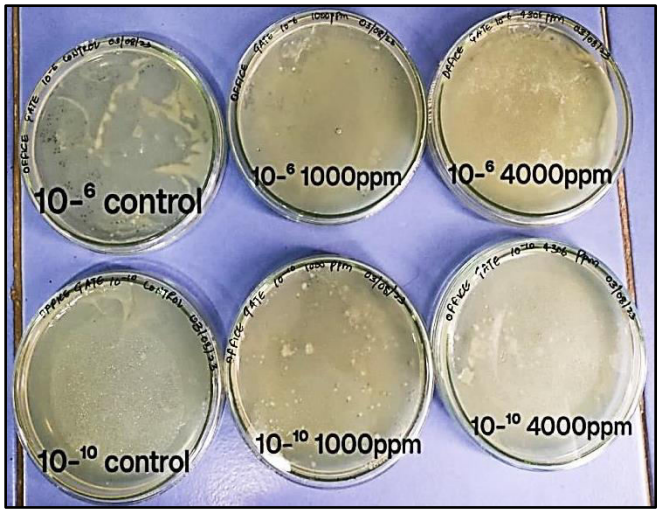


Fig. 47; OFFICE GATE

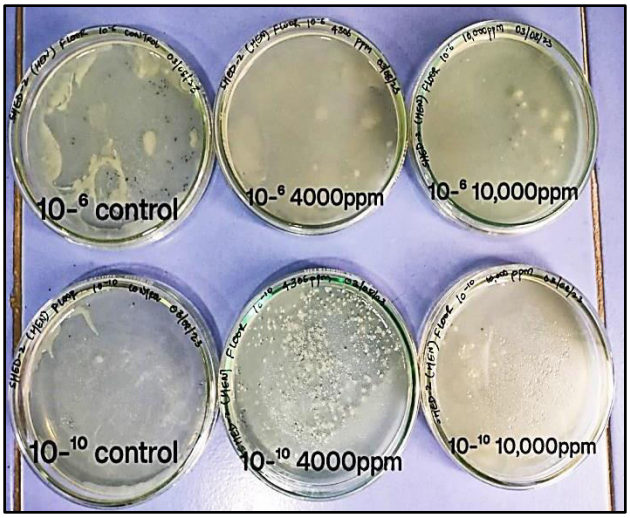


Fig. 50; SHED- 2 (HEN) FLOOR

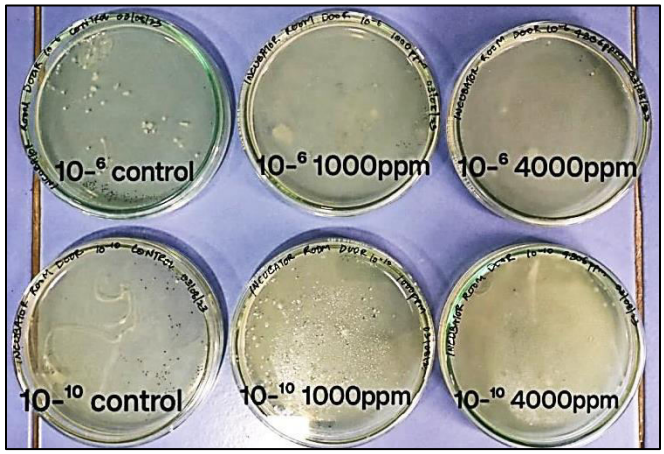


Fig. 48; INCUBATOR ROOM DOOR

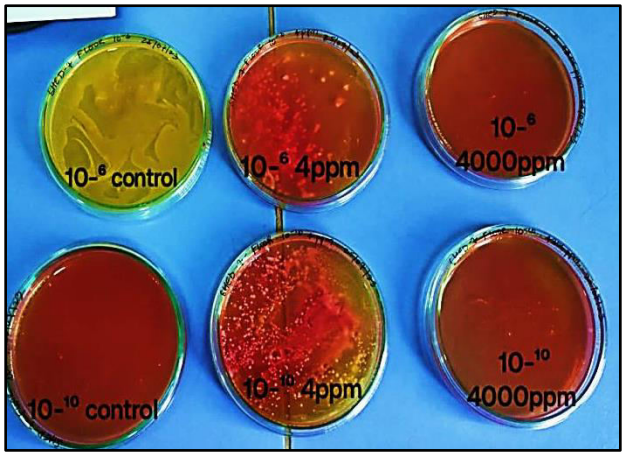


Fig. 51; SHED- 7 FLOOR

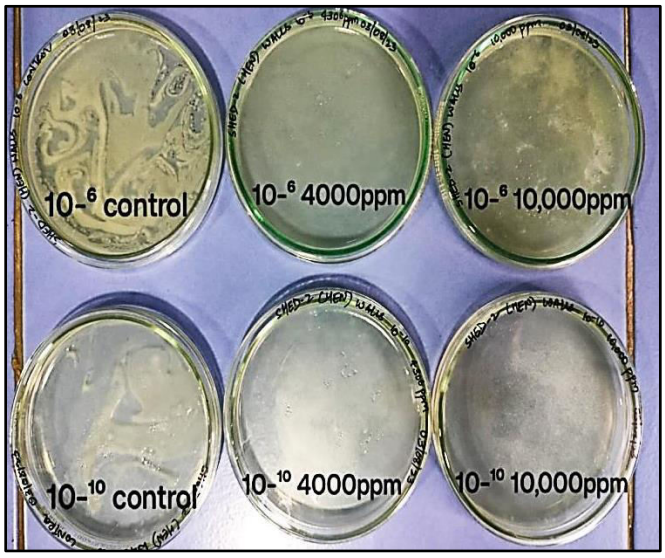


Fig. 49; SHED- 2 (HEN) WALLS

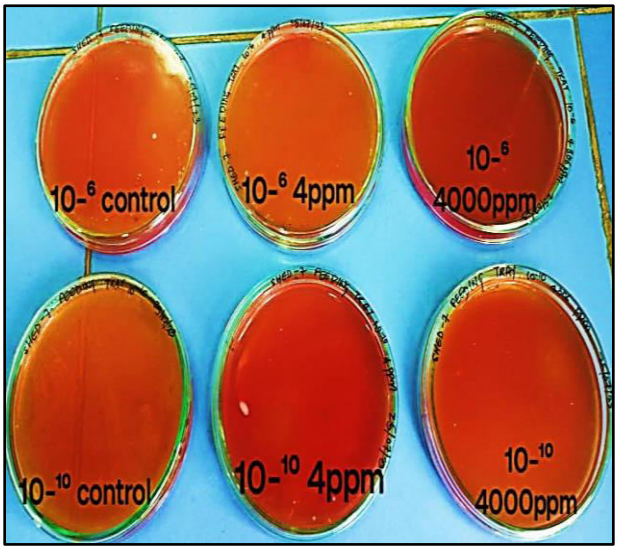


Fig. 52; SHED- 7 FEEDING TRAY

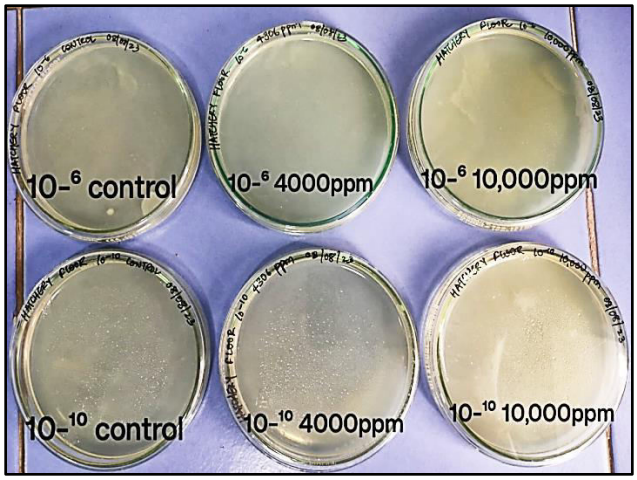


Fig. 53; HATCHERY FLOOR

Fig. 56; INCUBATOR ROOM HANDLE

2.2Against Fungi:

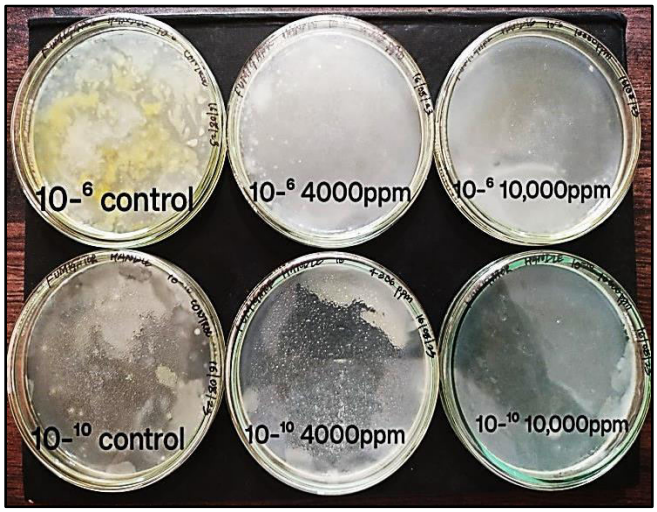


Fig. 54; FUMIGATOR HANDLE

Fig. 57; SHED- 7 WALLS

Fig. 55; FUMIGATOR FLOOR

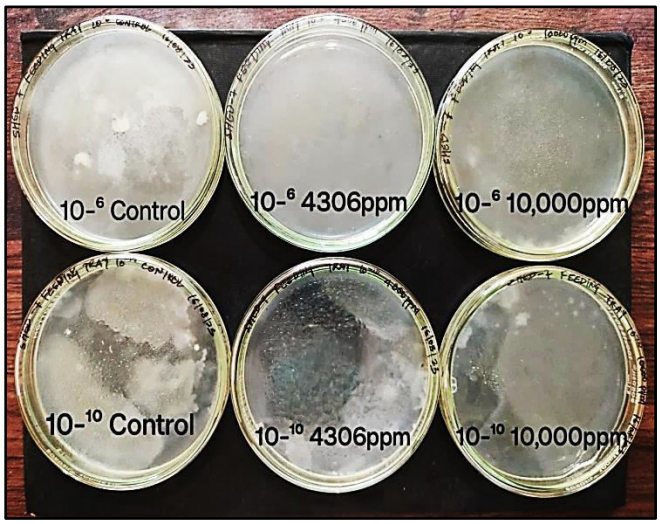


Fig. 58; SHED- 7 FEEDING TRAY

Fig. 59; SHED-2 (HEN) WALLS

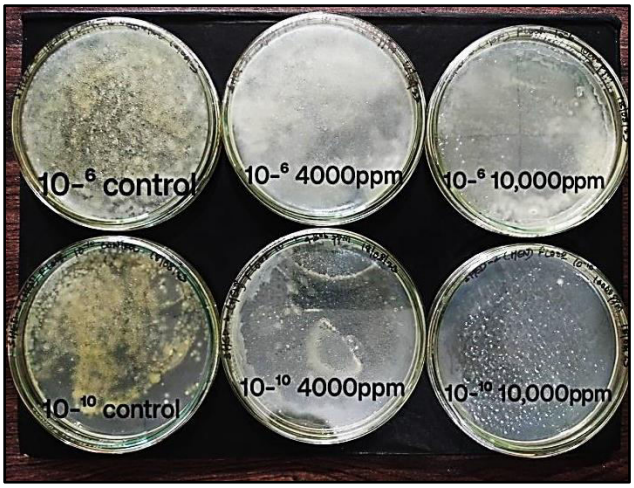


Fig. 62; SHED- 2 (HEN) FLOOR

Fig. 60; EGG SURFACRE

Fig. 64; HATCHERY FLOOR

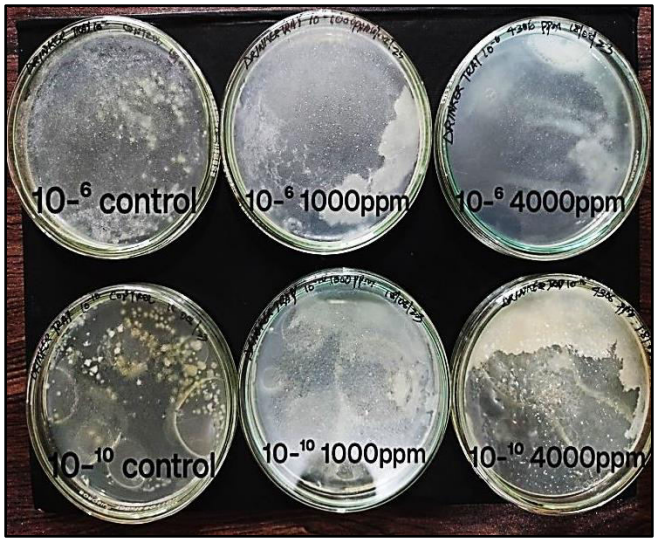


Fig. 61; DRINKER TRAY

Fig. 65; HATCHERY TRAY

Fig. 66; INCUBATOR ROOM DOOR

Fig. 69; OFFICE GATE

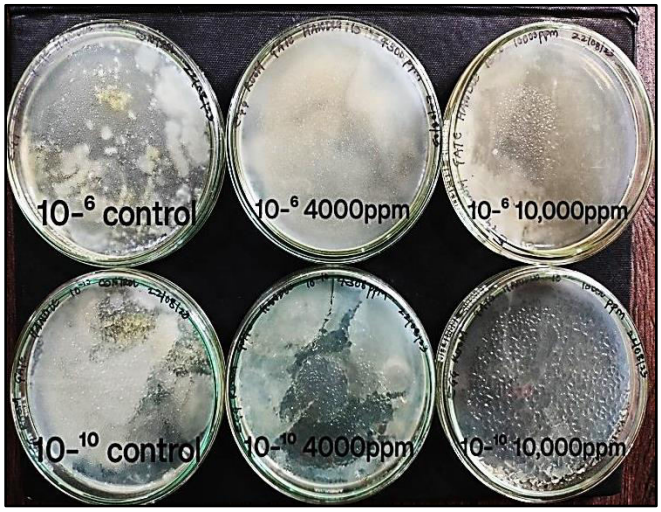


Fig. 67; EGG ROOM GATE HANDLE

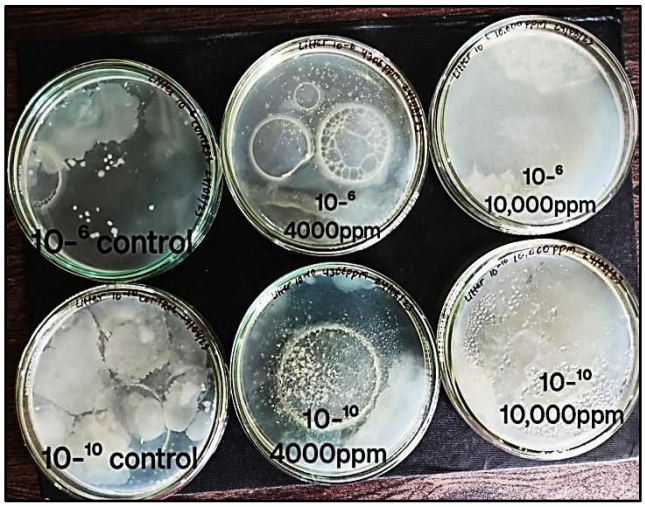


Fig. 70; LITTER

Fig. 68; MAIN GATE

3. COLONY FORMING UNIT (CFU):

1.1. For Bacteria:

Table 3; CFU of Bacterial Sample in CFU/ml

S. NO.	SAMPLE NAME	CONTROL CFU (in CFU/ml)		10^{-6} CFU at 1000 ppm (in CFU/ml)	10^{-10} CFU at 1000 ppm (in CFU/ml)	10^{-6} CFU at 4000 ppm (in CFU/ml)	10^{-10} CFU at 4000 ppm (in CFU/ml)	10-6 CFU at 10000 ppm (in CFU/ml)	10^{-10} CFU at 10000 ppm (in CFU/ml)
		10^{-6}	10^{-10}						
1.	Shed- 7 walls	195 x 10^{-6}	139 x 10^{-10}	-	-	Single diffused colony	88 x 10^{-10}	13x 10^{-6}	70x10^{-10}
2.	Shed- 7 feeding tray	200 x 10^{-6}	92 x 10^{-10}	-	-	8 x 10^{-6}	9 x 10^{-10}	NIL	NIL
3.	Shed- 7 floor	670 x 10^{-6}	428 x 10^{-10}	-	-	197 x 10^{-6}	128 x 10^{-10}	51x 10^{-6}	85 x 10^{-10}
4.	Fumigator floor	145 x 10^{-6}	60 x 10^{-10}	-	-	10x10^{-6}	4x10^{-10}	2x 10^{-6}	NIL
5.	Fumigator handle	142 x 10^{-6}	100 x 10^{-10}	-	-	81x10^{-6}	51 x 10^{-10}	61x 10^{-6}	NIL
6.	Incubator room door	117 x 10^{-6}	Single diffused colony	59 x 10^{-6}	24 x 10^{-10}	24 x 10^{-6}	NIL	-	-
7.	Incubator handle	75 x 10^{-6}	34 x 10^{-10}	65 x 10^{-6}	27 x 10^{-10}	38 x 10^{-6}	NIL	-	-
8.	Shed- 2 (hen) walls	164 x 10^{-6}	Single diffused colony	-	-	NIL	NIL	NIL	NIL
9.	Shed- 2 (hen) floor	136 x 10^{-6}	100 x 10^{-10}	-	-	31x10^{-6}	27 x 10^{-10}	14x 10^{-6}	5 x 10^{-10}
10.	Drinker tray	232 x 10^{-6}	102 x 10^{-10}	-	-	40 x 10^{-6}	NIL	NIL	NIL
11.	Hatchery tray	74 x 10^{-6}	190 x 10^{-10}	40 x 10^{-6}	53 x 10^{-10}	6 x 10^{-6}	6 x 10^{-10}	-	-
12.	Hatchery floor	77 x 10^{-6}	25 x 10^{-10}	-	-	NIL	NIL	NIL	NIL
13.	Egg room gate handle	100 x 10^{-6}	282 x 10^{-10}	-	-	41 x 10^{-6}	118 x 10^{-10}	NIL	94 x 10^{-10}
14.	Main gate	17 x 10^{-6} and a large diffused colony	25 x 10^{-10} and a large diffused colony	32 x 10^{-6}	26 x 10^{-10}	27 x 10^{-6}	27 x 10^{-10}	-	-
15.	Office gate	204 x 10^{-6}	106 x 10^{-10} and a large diffused colony	-	-	60 x 10^{-6}	92 x 10^{-10}	NIL	56 x 10^{-10}
		10^{-6} CFU Control (in CFU/ml)	10^{-10} CFU Control (in CFU/ml)	10^{-6} CFU at 100 ppm (in CFU/ml)	10^{-10} CFU at 100 ppm (in CFU/ml)	10^{-6} CFU at 200 ppm (in CFU/ml)		10^{-10} CFU at 200 ppm (in CFU/ml)	
16.	Egg surface	45 x 10^{-6}	51 x 10^{-10}	Cloudy appearance	12x10^{-10}	30 x 10^{-6}		NIL	

1.2. For Fungi:

Table 5; CFU of Fungi Sample in CFU/ml

S. NO.	SAMPLE NAME	CONTROL CFU (in CFU/ml)		10^{-6} CFU at 1000 ppm (in CFU/ml)	10^{-10} CFU at 1000 ppm (in CFU/ml)	10^{-6} CFU at 4000 ppm (in CFU/ml)	10^{-10} CFU at 4000 ppm (in CFU/ml)	10-6 CFU at 10000 ppm (in CFU/ml)	10^{-10} CFU at 10000 ppm (in CFU/ml)
		10^{-6}	10^{-10}						
1.	Shed- 7 walls	99 x 10^{-6}	54 x 10^{-10}	-	-	26 x 10^{-6}	15 x 10^{-10}	52 x 10^{-6}	12 x10^{-10}
2.	Shed- 7 feeding tray	126 x 10^{-6}	37 x 10^{-10}	-	-	NIL	NIL	NIL	NIL
3.	Shed- 7 floor	706 x 10^{-6}	428 x 10^{-10}	-	-	248 x10^{-6}	240 x 10^{-10}	NIL	NIL
4.	Fumigator floor	560 x 10^{-6}	83 x 10^{-10}	-	-	65 x10^{-6}	49 x10^{-10}	29 x 10^{-6}	80 x10^{-10}
5.	Fumigator handle	126 x 10^{-6}	171 x 10^{-10}	-	-	4 x10^{-6}	73 x 10^{-10}	NIL	NIL
6.	Incubator room door	160 x 10^{-6}	285 x 10^{-10}	52 x 10^{-6}	119 x 10^{-10}	37 x 10^{-6}	100 x 10^{-10}	-	-
7.	Incubator handle	137 x 10^{-6}	81 x 10^{-10}	44 x 10^{-6}	40 x 10^{-10}	63 x 10^{-6}	13 x 10^{-10}	-	-
8.	Shed- 2 (hen) walls	400 x 10^{-6}	96 x 10^{-10}	-	-	NIL	40 x 10^{-10}	NIL	NIL
9.	Shed- 2 (hen) floor	760 x 10^{-6}	400 x 10^{-10}	-	-	248 x10^{-6}	240 x 10^{-10}	240 x 10^{-6}	NIL
10.	Drinker tray	116 x 10^{-6}	149 x 10^{-10}	54 x 10^{-6}	96 x 10^{-10}	13 x 10^{-6}	19 x 10^{-10}	-	-
11.	Hatchery tray	40 x 10^{-6}	159 x 10^{-10}	32 x 10^{-6}	56 x 10^{-10}	NIL	NIL	-	-
12.	Hatchery floor	72 x 10^{-6}	103 x 10^{-10}	-	-	31 x 10^{-6}	41 x 10^{-10}	NIL	NIL
13.	Egg room gate handle	139 x 10^{-6}	129 x 10^{-10}	-	-	NIL	NIL	NIL	NIL
14.	Main gate	37 x 10^{-6}	225 x 10^{-10}	10 x 10^{-6}	11 x 10^{-10}	NIL	28 x 10^{-10}	-	-
15.	Office gate	46 x 10^{-6}	43 x 10^{-10}	-	-	34 x 10^{-6}	33 x 10^{-10}	NIL	NIL
		10^{-6} CFU Control (in CFU/ml)	10^{-10} CFU Control (in CFU/ml)	10^{-6} CFU at 100 ppm (in CFU/ml)	10^{-10} CFU at 100 ppm (in CFU/ml)	10^{-6} CFU at 200 ppm (in CFU/ml)		10^{-10} CFU at 200 ppm (in CFU/ml)	
16.	Egg surface	404 x 10^{-6}	364 x 10^{-10}	52 x 10^{-6}	116 x10^{-10}	43 x 10^{-6}		78 x 10^{-10}	

Discussion

The current study shows that adequate sanitation of the poultry farms, water quality, worker awareness, together with their hygiene habits, decreased the incidence of disease in chicken flocks and may enhance strategies for poultry production husbandry. In the upcoming years, demand for poultry, an efficient and generally healthy source of protein, is anticipated to rise quickly.

More bacteria and fungi were found in different parts of the chicken farm using microscopic identification, which led to the decision to employ an appropriate dose of Ef-Chlor for proper sanitation. Therefore, the time-kill method was used to test Ef-Chlor's antimicrobial activity against a variety of microbes detected in samples of poultry, demonstrating the product's effectiveness against different diseases. Therefore, a 99.99% reduction in CFUs was achieved.

In addition, maintaining the ideal temperature and humidity levels is essential for the survival of the poultry animals, which is why humidifiers were installed in sheds. Basic cleanliness is the most important factor in maintaining the health of chickens. Healthy chicks are mostly the result of healthy parents and clean hatcheries. Farm sanitation calls for using a reliable disinfectant and carrying out efficient cleaning with the suggested dosage of Ef-Chlor Bio tab/ Effi- Sept/ effi- Kleen 1.67gm and 4gm tablets and/or granules, as detailed in the tables.

As mentioned in the tables above, adequate washing with Ef-Chlor solution must be performed on dead chicken as well. The chicken is then properly covered, buried in a deep pit, and sprinkled with Ef-Chlor granules to prevent contamination.

Conclusion

On the basis of experiments performed, it can be concluded that Ef- Chlor Bio tab/ Effi- Sept/ effi- Kleen 1.67gm and 4gm tablets and/ or granules are effective against broad range of microorganisms.

To maintain a healthy environment in the poultry farm, daily hygiene and sanitation activities that begin with disinfection at the entrance of the farm to the hatchery area, correct washing method of sheds, and also burial of deceased chickens, should be properly observed. Therefore, it is believed that there would be a markedly reduced risk of infections in flocks of poultry when using the prescribed doses as listed in the directions for use tables.

Acknowledgments

- We appreciate Dr. Mahendra Singh and the staff at the poultry farm who kindly agreed to participate in the study. We especially appreciate them enabling us to share private information regarding their hygiene procedures.
- We would like to give a special thanks to Dr. Abhishek Gupta, director of the Center for Microbiology and Biotechnology (CMBT) Research and Training Institute, Nehru Nagar, Bhopal, Madhya Pradesh, 462003, for giving us the necessary time and space to complete the task on time.

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