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**Research Article**

# Efficiency of Some Sanitizers and Disinfectants against Biofilms and Planktonic Cells Buildup on Cages (Galvanized wire) and Plastic Material (PVC) in Poultry Farms

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**ABSTRACT**

This study aimed to evaluate the biofilmicidal efficiency of some sanitizers and disinfectant against *S. typhimurium* and *P. aeruginosa* biofilms which formed on the galvanized wire of cages and PVC of drinking lines in poultry farms and could hinder the efficacy of sanitation program. In addition, the effect of sanitizers on planktonic bacteria was studied. The results showed that on galvanized wire coupons after 10 min, only four sanitizers reduced *S. typhimurium* and *P. aeruginosa* biofilms count by 100%; Clorox 2.5%, Pril 2%+ Clorox 2.5%, Calcium hypochlorite 1% and Formalin 5%, While others were not sufficient to remove the biofilm completely. On PVC coupons after 24 hours, all used sanitizers removed *S. typhimurium* and *P. aeruginosa* biofilms completely except Iodocide 3%, Zix virox 0.2%, citric acid 1% and sodium hydroxide 1%. All used sanitizers and disinfectants showed bactericidal activity and removed the planktonic bacteria completely except sodium hydroxide 1%, and zix virox 0.2% which reduced the initial log by 62% and 50%, respectively. In conclusion, the presence of biofilms in the poultry environment is one of the most challenging problems and could lead to the failure of any biosecurity program particularly during disinfectant. Therefore, one step for removal of biofilms must precede the practice of disinfection operation. In our study, many sanitizers could achieve the required bacterial log reduction recommended by standards and were considered as bactericidal agent, but they are not suitable for use in the practical field as. Only sanitizers that remove the biofilm completely must be used to avoid opportunities for renewed attachment of bacteria and rapid establishment of a new biofilm.

**Key words:** Biofilmicidal, *S. typhimurium* biofilm, *P. aeruginosa* biofilm, Poultry farms

## INTRODUCTION

Egyptian poultry industry has been endangered by lots of variant strains of bacteria and viruses etc. Various types of vaccination programs have been designed for broiler, layer and parent stock folks to reduce mortality and morbidity. On the other hand, new biosecurity programs have been practiced for reducing bacterial and viral shedding that may cause a problem in the farm or surrounding farms. The backbone of any biosecurity practice depends on disinfectants and sanitizers efficient application. One of the common problems during sanitation program is the presence of biofilms which develop in water lines, drinking cups, bell drinkers, water drought, nipples, floors, walls, feeding augers, soils and poultry cages. Biofilms function as reservoirs for diverse species of bacteria, provide specific, limited niches and a protective refuge from competitors, predators, or harsh environmental conditions (Korber *et al.*, 1995). Pathogenic bacteria, e.g. *P. aeruginosa* and *S. typhimurium*, which attach readily to surfaces (Helke *et al.*, 1993), may establish in biofilms and detach along with pieces of biofilm into the environment. So, biofilm may hinder the efficiency of disinfectants and sanitizers during application.

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Biofilms are considered the most rich and widespread life form of bacteria, since biofilms enhance resistance of cells to the environmental stresses and defend cells against disinfectants and sanitizers (Hall-Stoodley *et al*., 2004). Biocides efficacy differs with cells of a planktonic and biofilm mode as many of them are effective against planktonic cells but not against biofilm cells (Kim *et al*., 2008). The mechanism of biofilm resistance to antimicrobials is not yet elucidated, because the mechanism of biofilm-associated antimicrobial resistance could be influenced by a lot of factors, and may vary from organism to organism. Association of bacteria in biofilm type structures increases the resistance to antimicrobial substances (Claudia *et al*., 2012).

Effective cleaning and disinfection routines are dependent on expert knowledge and experience. Removal of the attached bacteria and biofilm are difficult, and the design of attached cells is more resistant to biocides than planktonic cells, and the polysaccharide matrix provides a protective barrier limiting the penetration of disinfectants. When designing cleaning strategies, it is therefore important to consider the unique features of biofilms (Costerton *et al*.,1995). On the other hand, it is not sufficient to kill the microorganisms in the biofilm. The remaining polymer matrix must also be removed as it provides excellent opportunities for renewed attachment of bacteria and rapid establishment of a new biofilm (Wirtanen1995). The cleaning and disinfecting programme should be performed as cost-effectively and safely as possible, which means as infrequently as possible, in the shortest possible time, with low chemical, energy and labor costs, producing as little waste as possible and with no damage to the equipment (Lelieveld 1985; Holah 1992). Several chemical agents are commercially available for elimination of Salmonella and Pseudomonas aeruginosa. However, different studies revealed that cleansing and disinfection methods were ineffective against S. *typhimurium* in a field situation (Ramesh *et al*., 2002, Rose *et al*., 2000) in broiler houses. Parks *et al*. (2012) stated that, treatment with peracetic acid was more effective than treatment with sodium hypochlorite for inactivating *Salmonella* biofilm cells formed on stainless steel and PVC while Hallam *et al.,* (2001) proved that chlorine is a potent disinfectant to control biofilm growth.

The increasing resistance of *Pseudomonas* biofilms to different disinfectants was reported by numerous papers (Bjarnsholt *et al.,* (2007), Smith, K.; and Hunter, I.S (2008), Hendry *et al.,* (2009), and Tote *et al*., (2010).

This study aimed to evaluate the Biofilmicidal efficiency of some sanitizers and disinfectant against *S. typhimurium* and *P. aeruginosa* biofilms which formed on the galvanized wire of cages and PVC of drinking lines in poultry farms and could hinder the efficiency of sanitation program. In addition, the effect of sanitizers on planktonic bacteria will be studied.

## MATERIALS AND METHODS

### Bacterial cultures and cell suspension

Reference strain *S. typhimurium* ATCC@14028, and reference strain *P. aeruginosa* ATCC@9027 were inoculated into 9 ml of buffered peptone water (BPW (Lab M)). Reconstituted cultures were incubated at 37 °C for 24 h. One ml of the overnight culture was inoculated into a test tube containing nine ml tryptic soy broth, diluted 1:10 (Tryptic soy broth TSB (Lab M)). On the other hand, test water for planktonic bacterial growth was collected from a poultry farm that uses underground water to simulate natural life conditions. To characterize the type of water used (Table 1), it was microbiologically examined for determination of total bacterial count according to (APHA., 1984), detection of *S. typhimurium according* to ISO 6579 2002, detection of *E. coli* according to Lee and Arp 1998, and detection of *P. aeruginosa* according to Buxton and Fraser 1977. Chemical examination was performed according to AOAC 2015.

**Table 1:** Microbiological and Chemical examination of the tested water

| Test water parameters | Result |
|---|---|
| Total bacterial count | $25\times10^7$ cfu/ml |
| *Salmonella* | Negative |
| *E. coli* | Negative |
| *Pseudomonas* | Negative |
| pH | 7.2 |
| Magnesium | 13 mg/L |
| Calcium Carbonate | 100 mg/L |
| Magnesium Carbonate | 45 mg/L |
| Sulphates | 30mg/L |
| Chloride | 48 mg/L |
| Ammonia | Nil |
| Nitrite | Nil |
| Phosphorus | Nil |
| Phosphate | Nil |
| Total Hardness | 145mg/L |

### Preparation of galvanized wire and PVC coupons:

Galvanized wire coupons of poultry farm's cage material were prepared. The diameter of coupon was 2×3 cm. All coupons were sterilized by autoclaving at 121 °C for 15 minutes. A template was used in sampling procedure of one cc square area. Sterile PVC coupons of dimension 2.5 cm long and internal diameter of 1.90 cm were used. Preparation of coupons was performed according to Maharjan *et al.*, 2016.

### Biofilm and plankton growth formation

*S. typhimurium* and *P. aeruginosa* biofilms were prepared according to Ramesh *et al*., 2002. Briefly, sterile galvanized wire or PVC coupons were aseptically inserted into beakers containing 100 ml of *S. typhimurium* or *P. aeruginosa* culture. The beakers were incubated at 37 °C for 24h. Within a day, *S. typhimurium* and *P. aeruginosa* attached to the coupon surfaces. The coupons were gently removed from culture media. An aerobic plate count was performed to determine the concentration of the initial inoculum. The presence of biofilm was confirmed by crystal violet 1% stain according to Head and Yu, 2004 (Fig. 1). Attachment of planktonic bacterial growth of the tested water on PVC was prepared according to Maharjan *et al.*, 2017.

### Antibacterial sanitizers and disinfectants treatment

Initial bacterial counts after formation of the biofilm and before application of the disinfectants were determined and the $\log_{10}$ was calculated. Every coupon was covered with 2ml of the diluted tested sanitizers as reported in Table

2. The sanitizer was kept in contact for ten minutes for galvanized wire coupons and 24 hours for PVC coupons. Then each coupon was scraped with a pipette, and the fluid was aspirated from the petri dish and jetted back onto the coupon three times to dislodge attached bacteria from the coupon. The fluid from Petri dish was collected into a single tube. Then the fluid was 10-fold serially diluted, resulting in dilutions from $10^1$ to $10^{10}$. One ml neutralizer (3% Tween 80 (Mp Biomedicalis), 0.3 Lethcine (Fisher chemicals), 1% Histidine (Fisher chemicals), 0.5% Sodium thiosulphate (Fisher chemicals), 3% Saponine (Fisher chemicals)) according to Douglas and Kampf (2010) was prepared and added to the first dilution to inactivate the chemical compounds of the sanitizers and stop its effect. Bacterial re-isolation was made using specific medium; XLD (Lab M) for *Salmonella, P. aeruginosa* agar for *P. aeruginosa* and plate count agar for aerobic bacteria count. The bacterial counts were determined after application of the sanitizers for the desired contact time and the $\log_{10}$ was calculated. The difference between the before and after logs was the log reduction. According to European Committee for Standardization (CEN), *EN 13697-2001*, bactericidal activity was defined as $\geq 4 \log_{10}$ reduction of organisms attached to a surface under examination.

## RESULTS

The average initial count of *S. typhimurium,* for galvanized wire coupon, was 10.3 $\log_{10}$ CFU/ml. Table 3 showed (100%) log reduction of *S. typhimurium count* on tested galvanized wire coupon by Clorox 2.5%, Pril 2%+ Clorox 2.5%, Calcium hypochlorite 1% and Formalin 5%. While (90%)log reduction resulted from using Cupper sulphate, 1%, Dettol (25 ml / L) and Dyne O might (1part:400part). Regarding to Halamid 1%, Virkon S 1%, NaoH 1%, Formic acid1%, Ariel 5%, Citric acid 1%, Sulphmic acid 1% and Paraformaldehyde 1% showed bactericidal activity with log reduction ranged from 5.7 - 4.1 $\log_{10}$ CFU/ml ranging from 55%-40 %log reduction. While Synrgize (1part:256part), Ground Zero 0.5%, Zix virox 1%, acetic acid 1%, clean zix 7%, Iodocide 3% and Pril 2% did not show bactericidal activity with only log reductions ranged from 3.4 -1 $\log_{10}$ CFU/ml.

**Table 2:** Sanitizers used for biofilm removal

| Disinfectant | Active principle | Concentration | Company | Coupon used for |
|---|---|---|---|---|
| Acetic acid | Acetic acid 99% | 1% | Egyptian Petroleum Research Institute | galvanized wire and PVC |
| Citric acid | Citric acid 99% | 1% | Applichem chemicals | galvanized wire and PVC |
| Formic acid | Formic acid 99% | 1% | Fisher chemicals | galvanized wire and PVC |
| Sulphmic acid | Sulphmic acid 99% | 1% | Fisher chemicals | galvanized wire and PVC |
| Zix Virox | A synergetic combination of hydrogen peroxide 25%, peracetic acid synergized 5%, organic acids, stabilizers and surfactants | 1% | Biocidas biodegradable zix(Spain) | galvanized wire and PVC |
| Clean Zix | A combination of alkalis with surfactants, wetting agents, emulsifiers and sequestrants. | 7% | Biocidas biodegradable zix(Spain) | galvanized wire and PVC |
| Iodocide | Nonylphenoxypolyethoxyethanol - Iodine complex 2.3%-Poly(oxy-1,2-ethanediyl), .alpha.-(nonylphenyl)-.omega.-hydroxy-, compd. with iodine 2.3% | 3% | Range Pharma SDN (Malysia) | galvanized wire and PVC |
| Dettol | Chloroxylenol ($C_8H_9C_{lO}$), comprises 4.8% of Dettol's total admixture, with the rest made up by pine oil, isopropanol,castor oil, soap and water. | 2.5% | Royal Cosmoetic Co. for Reckitt Benckiser | galvanized wire only |
| Synrgize | Glutaraldehyde (7%) and Quaternary Ammonium (26%). | 0.39% | Preserve (USA) | galvanized wire only |
| Dyne O might | Iodine 0,42% inert ingredients 99,58% Total 100,00% *From Polyoxyethylene-polyoxypropylene block polymer Iodine Complex | 0.25% | Preserve (USA) | galvanized wire only |
| Virkon S | potassium peroxymonosulfate | 1% | DuPont | galvanized wire and PVC |
| NaoH | NaoH 99% | 1% | Loba chemie | galvanized wire and PVC |
| Halamid | 24.4% Chlorine | 1% | Axcentive Sarl. | galvanized wire and PVC |
| Pril | Non-ionic surfactants (5%) and anionic surfactants (5-15%). | 2% | Henkel factory production | galvanized wire only |
| Clorox | Sodium hypochlorite less than 5% | 2.5% | Egyptian Company for Detergents production | galvanized wire and PVC |
| Pril+ Clorox | Non-ionic surfactants (5%) and anionic surfactants (5-15%) + Sodium hypochlorite less than 5% | 2%+ 2.5%, | Egyptian Company for housholding detergents | galvanized wire only |
| Formalin | Formaldehyde 37% | 5% | El Salam for Chemicals Industries | galvanized wire only |
| Paraformaldehyde | Formaldehyde 98% | 1% | Loba chemie | galvanized wire only |
| Cupper Sulphate | Cupper sulphate 99% | 1% | Fisher chemicals | galvanized wire and PVC |
| Ground Zero | 3% Iodophre-3.5% glutrldehyde (Acid base) | 0.5% | Electrostatics Bradenton | galvanized wire and PVC |
| Calcium hypochlorite | Calcium hypochlorite 89% | 1% | Egyptian company for chemicals production | galvanized wire and PVC |
| Ariel | Protease (enzyme), 5-15% Anionic surfactant nonionic surfactant, <5% phosphantes | 5% | Procter and Gomble Amiens | galvanized wire only |

The average initial count of *P. aeruginosa*, for galvanized wire coupon, was 9 $log_{10}$ CFU/ml. Table (3) showed the log reduction 100% of *P. aeruginosa* count on tested galvanized wire coupon. On the addition of Clorox 2.5%, Pril 2%+ Clorox 2.5%, Calcium hypochlorite 1%, Formalin While 89% log reduction could be obtained form %, Dettol (25 ml per Liter), Dyne O might (1part:400part) and Copper Sulphate 1%. While Sodium hydroxide 1%, Synergize (1part:256part) and Formic acid1%, bactericidal activity was recorded with log reduction of 9 - 4.1 $log_{10}$ CFU/ml. While Halamid 1%, Virkon S 1%, Ariel 5%, Sulphamic acid 1%, Citric acid 1%, clean zix 7%, Zix virox 1%, acetic acid, Paraformaldehyde 1%, Iodocide 3%, Ground Zero 0.5% and Pril 2% showed no bactericidal activity with log reduction ranged from 3.8 - 0 $log_{10}$ CFU/ml.

The average initial count of *S. typhimurium*, for PVC coupon, was 11 $log_{10}$ CFU/ml. Table (4) showed the log reduction of *S. typhimurium* on tested PVC coupon. Almost all tested sanitizers showed bactericidal activity against *S. typhimurium* biofilm with log reduction ranged from 11 – 10 $log_{10}$ CFU/ml. Zix Virox 0.2% was the only sanitizer that did not show bactericidal activity with log reduction of only 2 $log_{10}$ CFU/ml.

The average initial count of *P. aeruginosa*, for the PVC coupon, was 10.1 $log_{10}$ CFU/ml. Table (4) showed the log reduction of on tested PVC coupon. As *S. typhimurium* biofilm, almost all tested sanitizers showed bactericidal activity against *P. aeruginosa* biofilm with log reduction ranged from 10.1 – 4.7 $log_{10}$ CFU/ml. Zix Virox 0.2% again was the only sanitizer that did not show bactericidal activity with log reduction of only 0.1 $log_{10}$ CFU/ml.

The average initial count of planktonic bacterial growth on the PVC coupon was 8 $log_{10}$ CFU/ml. The results in Fig. 2 showed the log reduction percentage of planktonic bacterial growth on tested PVC coupon. All used sanitizers; copper sulphate 1%, Halamid 1%, Virkon S 1%, Formic acid1%, acetic acid 1%, Sulphamic acid 1%, Citric acid 1%, Clorox 2.5%, Calcium hypochlorite 1%, Iodocide 3%, clean zix 7% and Dyne O might (1part:400part) showed bactericidal activity and removed the planktonic bacteria completely except sodium hydroxide 1%, and zix virox 1% which reduced the initial log by 62% and 50%, respectively.

## DISCUSSION

This study aimed to evaluate the efficiency of some disinfectants and sanitizers in reducing population of *S. typhimurium* and *P. aeruginosa* biofilms which formed on the galvanized wire of cages and PVC of drinking lines in poultry farms.

Table 3 shows the efficiency of sanitizers against *S. typhimurium* and *P. aeruginosa* biofilms on galvanized wire coupons after 10 min. Only four sanitizers reduced *S. typhimurium* and *P. aeruginosa* biofilms count by 100%; Clorox 2.5%, Pril 2%+ Clorox 2.5%, Calcium hypochlorite 1% and Formalin 5%. These results agree with Moretro *et al.,* (2009) who mentioned that hypochlorite was more efficient for biofilm removal. Also, Fernanda *et al.,* (2015) recorded that chlorine-based disinfectant agent decreased *P. aeruginosa* biofilm within the space of 5 minutes. On the other hand, our results disagree with Norwood and Gilmour (2000) who stated that microbial biofilms present

**Table 3:** Efficiency of sanitizers against *Salmonella* and *Pseudomonas* biofilms on galvanized wire coupons after 10 min. contact time.

| Disinfectant | *Salmonella*[a] | | *Pseudomonas*[b] | |
|---|---|---|---|---|
| | log reduction | Log reduction percentage (%) | log reduction | log reduction percentage (%) |
| Clorox | 10.3 | 100% | 9 | 100% |
| Pril+Clorox | 10.3 | 100% | 9 | 100% |
| Calcium hypochlorite | 10.3 | 100% | 9 | 100% |
| Formalin | 10.3 | 100% | 9 | 100% |
| Copper sulphate | 9.3 | 90% | 8 | 89% |
| Dettol | 9.3 | 90% | 8 | 89% |
| Dyne O might | 9.3 | 90% | 8 | 89% |
| Halamid | 5.7 | 55% | 3.8 | 42% |
| Virkon S | 5.3 | 51% | 3.7 | 41% |
| NaoH | 5.3 | 51% | 5 | 56% |
| Formic acid | 5.3 | 51% | 4.1 | 46% |
| Ariel | 4.7 | 45% | 3.3 | 36% |
| Citric acid | 4.7 | 45% | 2.6 | 29% |
| Sulphmic acid | 4.5 | 43% | 2.8 | 31% |
| Paraformaldehyde | 4.1 | 40% | 1.6 | 17% |
| Synrgize | 3.4 | 33% | 4.7 | 52% |
| Ground zero | 3.2 | 31% | 1 | 11% |
| Zix Virox | 3.2 | 31% | 1.7 | 19% |
| Acetic acid | 3 | 29% | 2 | 22% |
| Clean Zix | 3 | 29% | 2.2 | 24% |
| Iodocide | 2.9 | 28% | 1.1 | 12% |
| Pril | 1 | 9.7% | 0 | 0% |

[a]*Salmonella* population log before treatment was 10.3 expressed as A, *Salmonella* population log after treatment expressed as B, *Salmonella* log reduction=A-B, *Salmonella* reduction Percentage (%) = (A-B/A) ×100; [b]*Pseudomonas* population log before treatment was 9 expressed as A, *Pseudomonas* population log after treatment (expressed as B), *Pseudomonas* log reduction (A-B), *Pseudomonas* reduction Percentage (%) = (A-B/A) ×100.

**Table 4:** Efficiency of sanitizers against *Salmonella* and *Pseudomonas* biofilms on PVC coupons after 24 hours contact time.

| Disinfectant | *Salmonella*[a] | | *Pseudomonas*[b] | |
|---|---|---|---|---|
| | Log reduction | Log reduction percentag (%) | Log reduction | Log reduction Percentage (%) |
| Copper sulphate | 11 | 100% | 10.1 | 100% |
| Halamid | 11 | 100% | 10.1 | 100% |
| Virkon S | 11 | 100% | 10.1 | 100% |
| Formic acid | 11 | 100% | 10.1 | 100% |
| Acitic acid | 11 | 100% | 10.1 | 100% |
| Sulphmic acid | 11 | 100% | 10.1 | 100% |
| Clorox | 11 | 100% | 10.1 | 100% |
| Calcium hypochlorite | 11 | 100% | 10.1 | 100% |
| Clean Zix | 11 | 100% | 10.1 | 100% |
| Dyne O might | 11 | 100% | 10.1 | 100% |
| NaoH | 10 | 90% | 8.1 | 80% |
| Iodocide | 10 | 90% | 6.5 | 64% |
| Citric acid | 11 | 100% | 4.7 | 46% |
| Zix Virox | 2 | 18% | 0.1 | 1% |

[a]*Salmonella* population log before treatment was 11 expressed as A, *Salmonella* population log after treatment expressed as B, *Salmonella* log reduction=A-B, *Salmonella* reduction Percentage (%) = (A-B/A) ×100; [b]*Pseudomonas* population log before treatment was 10.1 expressed as A, *Pseudomonas* population log after treatment (expressed as B), *Pseudomonas* log reduction (A-B), *Pseudomonas* reduction Percentage (%) = (A-B/A) ×100.

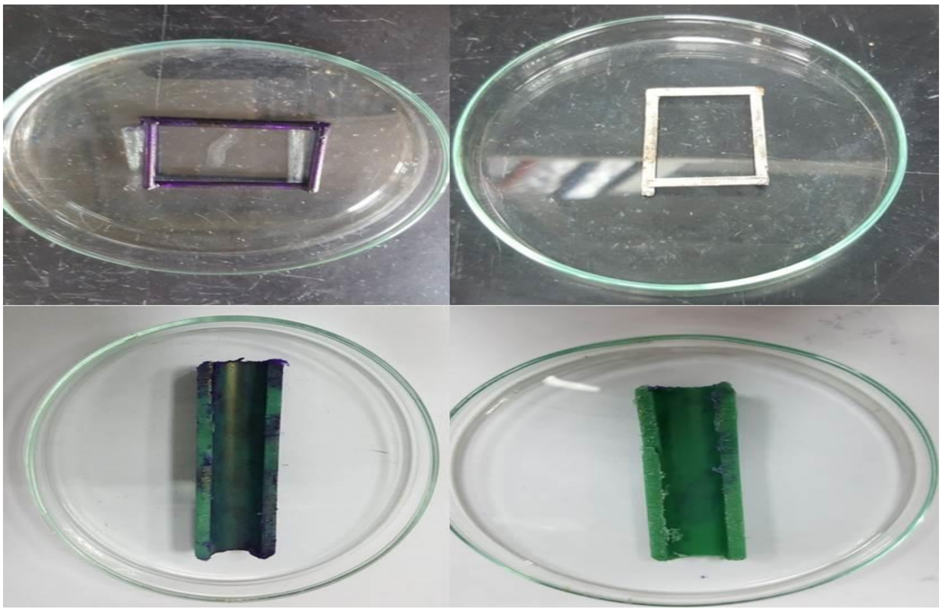

**Fig 1:** GGalvanized wire and PVC coupons; clean on upper and lower right, and with gentian violet-stained biofilms on upper and lower left.

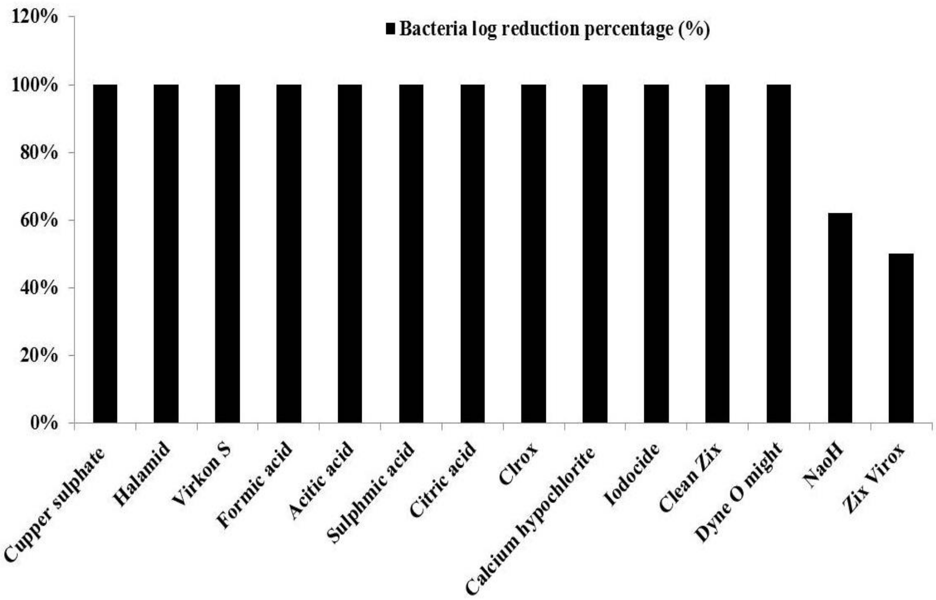


**Fig 2:** The effect of different sanitizers on log reduction percentage of planktonic bacterial growth on tested PVC coupon.

great resistance to active chlorine. Also, our results disagree with (Moretro *et al.,* 2009 and Corcoran *et al*., 2014) who reported that hypochlorite and benzalkonium chloride didn't achieve eradication of biofilm, even at the 90-min contact time. Regarding to Formalin 5%, we disagree with Gradel *et al.,* (2004) who reported formaldehyde was more effective than glutaraldehyde in field conditions but did not guarantee total *S. typhimurium* elimination.

Dyne O might (iodine) reduced *S. typhimurium* log count by 9.3 (90%). This result disagree with Ronner and Wong (1993) who mentioned that iodine, chlorine, a quaternary ammonium compound, and an anionic acid generally caused 3-5 log reduction of biofilm *S. Typhimurium*, on stainless steel.

Virkon S reduced *S. typhimurium* log count by 5.3 (51%). This result agree with Møretrø *et al.,* (2012) who found that the exposure to acidic peroxygen- based disinfectants (Virkon S) resulted in the 4 $\log_{10}$ reduction in *S. typhimurium* cells.

Synrgize (glutaraldehyde) was not sufficient to remove the biofilm completely, this result agree with Marin *et al.,* (2009) who conducted that the use of glutaraldehyde at a concentration of 1.0% was insufficient to remove *S. typhimurium from* the poultry houses in field conditions.

Zix virox (peracetic acid) reduced the log count by 3.2 and 1.7 for *S. typhimurium* and *P. aeruginosa*, respectively. This result agrees with Stewart (2000) who proved that the biofilm formed by *P. aeruginosa* could be reduced to 1 log cycles by peracetic acid. However, our results disagree with Simone *et al.,* (2007) who showed that peracetic acid reduced bacterial count of biofilm formation to 5.26 decimal reductions for adhered cells on stainless steel.

Regarding to Pril® (cationic and anionic surfactants), the current results agree with Adair *et al.,* (1969) who mentioned that cationic surfactants are not very active against *P. aeruginosa*. The addition of Pril to Clorox didn't affect the good efficacy of Clorox, but in the field it could be a good combination for the removal of organic matter by Pril and the eradication of *S. typhimurium* and *P. aeruginosa* by clorox.

Although copper sulphate, Dettol, Dyne O might, Halamid, Virkon S, NaoH and Formic acid had bactericidal activity and efficiently reduced ≥ 4 $\log_{10}$ of both *S.*

*typhimurium* and *P. aeruginosa* biofilms, but no complete elimination of biofilm was achieved which is the main aim of sanitation program. Therefore, we recommend the use of Clorox 2.5%, Pril 2%+ Clorox 2.5%, Calcium hypochlorite 1% or Formalin 5% for elimination of *S. typhimurium* and *P. aeruginosa* biofilms from galvanized wire poultry cages.

Table 4 shows the efficiency of sanitizers against *Salmonella* and *Pseudomonas* biofilms on PVC coupons after 24 hours. All used sanitizers removed *S. typhimurium* and *P. aeruginosa* biofilms completely except Iodocide 3%, Zix virox 0.2%, citric acid 1% and sodium hydroxide 1%. These results agree with Hallam *et al.,* (2001); Butterfield *et al.,* (2002) who proved that chlorine is a potent disinfectant to control biofilm growth compared to hydrogen peroxide. On the contrary, a high efficacy of chlorinated sanitizers is needed to control *P. aeruginosa* especially when other studies confirm that biofilm bacteria of *P. aeruginosa* are less susceptible to chlorine than planktonic counterparts (Cochran *et al.,* (2000); Kim *et al.,* (2008). Single cells that may remain viable in biofilms even though a bactericidal treatment was applied are significant hazardous factors for new biofilm formation and biosecurity breach. Hence, only sanitizers with complete removal of biofilms should be used for water lines.

A sample of underground, untreated water was examined from poultry farm. The microbiological and chemical parameters of the tested water are shown in table (1). The bacterial count which recovered on the PVC coupons after 7 days was 8 $\log_{10}$ CFU/ml. All used disinfectants removed the planktonic bacteria completely except NaoH and zix virox. These results agreed with Maharjan *et al.,* (2016) who mentioned that chlorine based sanitizers can help mitigate already formed plankton. Also, Magdalena *et al.,* (2016) mentioned that chlorine-based and quaternary ammonium compound-based has a great effect on the planktonic bacteria.

It is worthy to mention, biofilmocidal effect of sanitizers varies according to the surface they act upon and the type of microbe they involved with. In our study, only clorox 2.5% and calcium hypochlorite 1% possessed the Biofilmicidal effect against *S. typhimurium* and *P. aeruginosa* biofilms on both galvanized wire of cages and PVC of drinking lines. The well-known capability of chlorine based sanitizers to inactivate pathogens in suspensions, and its low cost contribute to its common usage (Luo *et al.,* 2011).

In conclusion, the presence of biofilms in the poultry environment is one of the most challenging problems and could lead to the failure of any biosecurity program. Therefore, a step for the removal of biofilms must be added before the procedure of disinfection of poultry house. In our study, many sanitizers could achieve the required bacterial log reduction recommended by standards and were considered as bactericidal agent, but they are not suitable for use in the practical field. Only sanitizers that remove the biofilm completely must be used to avoid opportunities for renewed attachment of bacteria and rapid establishment of a new biofilm.

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