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Evaluation of the Efficacy of Some Biocides to Reduce Common Microbial Species: Total Aerobes, Yeast and Molds.

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ABSTRACT

The current study aimed to evaluate six types of commercial disinfectants available in the local markets and the most commonly used at the farm level within poultry flocks in Egypt in terms of their ability to stop common microbial species (total aerobes, yeast, and mold) and their resistance through different methods of application (Low rate Spray, High rate Spray, Cold Fogging). It is utmost significant to remember that for the field trials ,all surface wastes and loose animate matter had been removed before disinfectant application. Method and rate of application are very important to obtain efficient disinfection process as well as complete destroying of pathogens or even minimizing the level to ensure the prevention of the infection. A study was carried out at faculty of veterinary medicine Cairo University, the disinfectants Formalin, QACs, Phenol, Virkon-S, Halamid, and Micro-Sept M were chosen. The results showed that: A) At low application rates, disinfectant treatments had no noticeable effect on total aerobic bacterial populations excluding Formalin and Virkon-S, where the decrease in log 10 was 2 and 1.7, respectively compared with the control group). B) High application rates of selected disinfectants impacted and affect significantly the examined microorganisms. C) Cold fogging resulted in the greatest effect among other two above mentioned experiments on aerobic bacteria, yeast, and mold populations. Concerning, mold: Fogging resulted in a significant effect of selected disinfectants.

Keywords: Cold fogging, Disinfections, Layer Flocks, Microbial Load, Rate of Application.

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Air pollution with microorganisms in poultry houses is an important basis for biological hazards **(Whyte *et al.*, 2001).** Air plays an important function in the transmission of diseases and is involved in food contamination at many phases of processing **(Lues *et al.*, 2007)**. In intensive poultry production, broiler chickens in particular can be a significant source of air and surface pollution where dust, suspended dirt, faeces, feathers and skin fragments are a major cause of impact on animal health and public health hazards to breeders and people living around them. **(Donham, 1993).**

In intensive production systems, stocking density in poultry houses is incredibly high, this making it difficult to keep up optimal microclimate and sanitary conditions. The health status of both birds and personnel is full of air micro-flora. **(Vučemilo *et al.*, 2008).**

Several studies in poultry houses indicated that birds are a source of microbes, followed by feed, litter and excrement, and the numbers of these microbes are affected mainly by the efficiency of the ventilation system, which have health effects on animals and humans through exposure to molds and volatile mycotoxins.produced by them **(Herbut *et al.*, 1982; Kluczek *et al.*, 1993; Sikorska, 2006).**

There are epidemiological studies indicating high concentrations of air pollutants such as dust, gases, microorganisms and their toxins in a continuous manner that may harm the workers' health in animal houses. **(Whyte *et al.*, 1993).**

In broiler houses, hatcheries, and egg processing facilities, airborne microbial populations and aerosol formation have been studied **(Duan *et al.*, 2008; Ricke *et al.*, 2019).**

Many germs found in fowl litter and floor come from feces, such as Enterobacteriaceae and other zoonotic pathogens . These germs in fowl premises could be higher than at hatcheries, in addition, to count in-floor houses Litter nearly higher 9 times within the air than dwellings wire floor **(Quarle *et al.*, 1970; Fries *et al.*, 2005**). Airborne particles found at chicken farms include many viruses, bacteria, and fungi (**Zhang *et al.*, 1994; Martin *et al.*, 1996; Zhai *et al.*, 2020)**. The majority of authors have counted the total number of bacteria but only a small percentage of them have quantified and identified the genus and species (**Guillam *et al.*, 2007**).

Due to the lack of studies that indicate the efficiency of the work of some biocides available in the local markets, the current study aimed to give a clear picture of the efficiency of these disinfectants against microbial load on the floor of poultry houses through traditional and new application methods.

MATERIALS AND METHODS

Experimental design

Experimental units were six of 1-ft^2 square area (floor plots) , each of which separated by 1-ft^2 area and randomly blocked. between each experimental plots. Two test-units (2n) for each disinfectant were used. Negative controls were used in the treatments. Half samples were taken (a 5 × 2 factorial design), fifteen minutes after use while the other were tested 6 hours and 24 hours later.

Application of the disinfectants

Six treated plots for each disinfectant examined microbiologically (2 replicates per treatment):

- Coarse spray at low application level 50 ml/examined plot. The rate was chosen due to its ability to create a good surface coverage.
- As a spray at high application level 125 ml/examined plot. . The rate was chosen because it correlated to a common disinfectant usage level of 500 gal/16,000 ft^2 (Payne et al., 2005).
- Cold fogging at 125 ml/examined plot.

Aerobic Microbial Plating

Total Bacterial, Yeast and Mold Counts using 3MTM Petri-filmTM (U.S.Food and Drug Administration, 2001**).** Cellulose drag sponges were moistened with 20 ml Buffer Phosphate Diluents (BPD) and placed in sterile bags. Sponges were aseptically separated from each bag by a string and used to sample the plot's surface. To make a 1:10 dilution, each sponge was placed in180 ml BPD. The samples were immediately transported to the laboratory and kept cool using ice packs.

Table 1: Selected Disinfectants

Disinfectant	Manufacture	Concentration
Formalin	Egypt	4% (V/V)
Phenol	Egypt	5% (V/V)
QACs	Germany	Diluted 1:3
Halamid	USA	Diluted 1:18
Virkon-S	USA	1% (W/V) potassium peroxymonosulfate and sodium chloride in H_2o
Micro Sept M	USA	1:5 (for spraying) and Conc. (for fogging)

Using distilled water, each disinfectant was made according to the manufacturer's instructions.

Samples were serially diluted ten times up to the third dilution level for APC while the second dilution level for mold. The plating was done in duplicate for each dilution level and the median count was used (YM broth and agar were used) **(U.S. Food and Drug Administration, 2001).**

Statistical Analysis

One-way ANOVA was used to compare disinfectants after transforming the data to log10 values.. The comparison of variables with a significant F test was used and $P < 0.05$ was used to determine significance.

RESULTS

The effect of disinfectant application rate and contact time on total aerobic bacteria, yeast, and molds (Table 2):

Table 2: The effect of low application level and contact time on total aerobic bacteria, yeast, and molds:

Mic / Time	Bacterial Count			Yeast Count			Mold Count		
	15 min	6 hr.	24hr	15 min	6 hr.	24hr	15 min	6 hr.	24hr
Control	7.2 [a]	7.1 [a]	7.2 [a]	1.8 [a]	1.7 [a]	1.6 [a]	1.2 [a]	1.2 [a]	1.1 [a]
Formalin	5.2 [b]	5.1 [b]	5 [b]	1.2 [b]	1. 2 [b]	1.1 [b]	0.8 [b]	0.8 [b]	0.7 [b]
Phenol	6.2 [b]	6.2 [b]	6.1 [b]	1.4 [b]	1.3 [b]	1.1 [b]	1.1 [b]	0.9 [b]	0.9 [b]
QACs	7 [b]	7 [b]	6.8 [b]	1.6 [b]	1.6 [b]	1.5 [b]	1.2 [a]	1.1 [b]	1.1 [a]
Halamid	6 [b]	5.8 [b]	5.7 [b]	1.2 [b]	1.1 [b]	1.1 [b]	0.9 [b]	0.8 [b]	0.8 [b]
Virkon-S	5.5 [b]	5.4 [b]	5.2 [b]	1.6 [b]	1.4 [b]	1.3 [b]	0.7 [b]	0.6 [b]	0.55 [b]
Micro. Sept M	6.2 [b]	6.1 [b]	6.1 [b]	1.4 [b]	1.4 [b]	1.3 [b]	0.9 [b]	0.8 [b]	0.8 [b]

a-b Different superscripts mean significantly different column values (P<0.05). A 50 ml /plot.

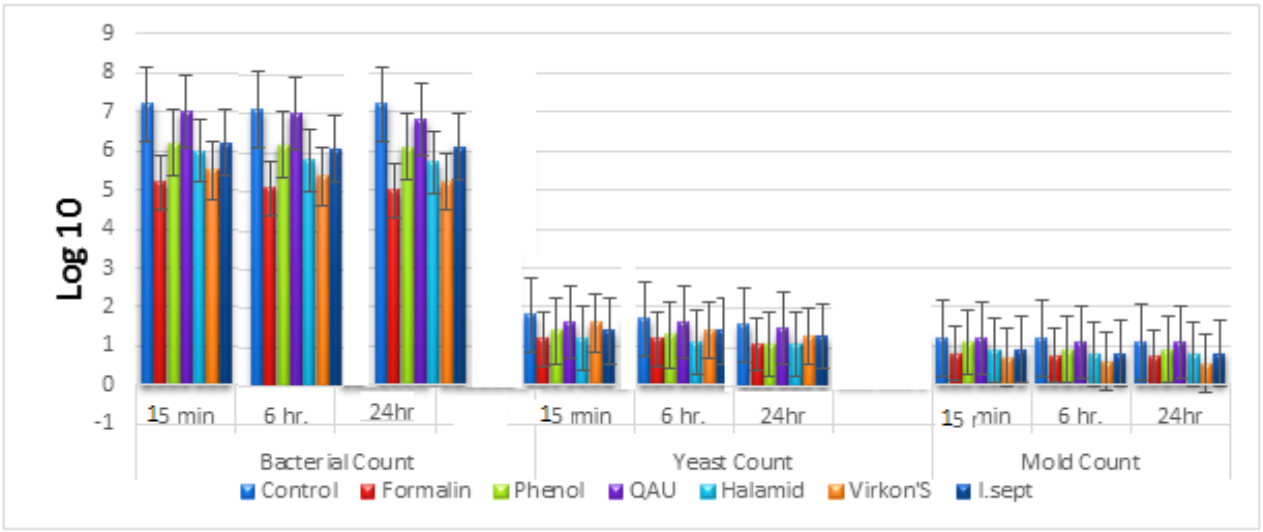

Fig. 1: The effect of low application level and contact time on total aerobic bacteria, yeast, and molds.

Table 3: The effect of high application level and contact time on total aerobic bacteria, yeast, and molds:

Mic / Time	Bacterial Count			Yeast Count			Mold Count		
	15 min	6 hr.	24hr	15 min	6 hr.	24hr	15 min	6 hr.	24hr
Control	7.2 [a]	7.1 [a]	7.2 [a]	1.8 [a]	1.7 [a]	1.6 [a]	1.2 [a]	1.2 [a]	1.1 [a]
Formalin	4.22 [b]	4.1 [b]	4.12 [b]	0.9 [b]	0.82 [b]	0.81 [b]	0.6 [b]	0.55 [b]	0.52 [b]
Phenol	5.2 [b]	5.2 [b]	5.1 [b]	1.2 [b]	1.0 [b]	0.9 [b]	0.7 [b]	0.6 [b]	0.6 [b]
QACs	6.2 [b]	6.2 [b]	6.1 [b]	1.4 [b]	1.4 [b]	1.3 [b]	0.91 [b]	0.82 [b]	0.81 [b]
Halamid	5 [b]	4.8 [b]	4.7 [b]	1.2 [b]	1.2 [b]	1.1 [b]	0.9 [b]	0.8 [b]	0.8 [b]
Virkon-S	4.5 [b]	4.4 [b]	4.2 [b]	1.2 [b]	1.1 [b]	1.1 [b]	0.7 [b]	0.6 [b]	0.55 [b]
Micro Sept M	4.2 [b]	4.1 [b]	4.1 [b]	1.2 [b]	1.2 [b]	1.1 [b]	0.9 [b]	0.8 [b]	0.8 [b]

a-b Different superscripts mean significantly different column values (P<0.05). A 125 ml/plot.

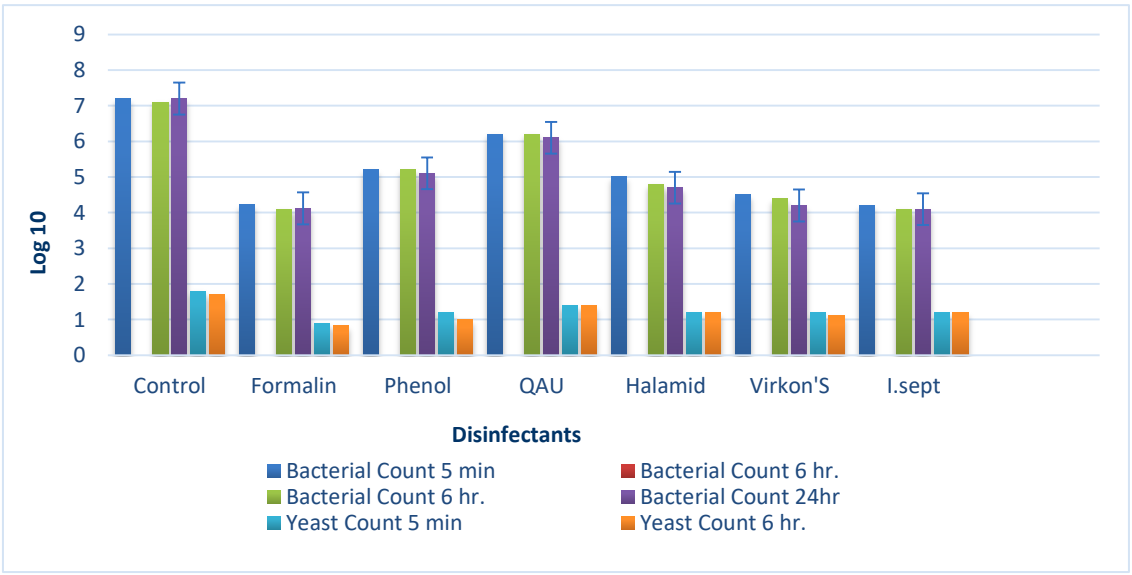

Fig. 2: The effect of high application level and contact time on total aerobic bacteria, yeast, and molds.

Table 4: The effect of cold fogging and contact time on total aerobic bacteria, yeast, and molds.

Mic / Time	Bacterial Count			Yeast Count			Mold Count		
	15 min	6 hr.	24hr	15 min	6 hr.	24hr	15 min	6 hr.	24hr
Control	7.2^{a}	7.1^{a}	7.2^{a}	1.8^{a}	1.7^{a}	1.6^{a}	1.2^{a}	1.2^{a}	1.1^{a}
Formalin	3.2^{b}	3.1^{b}	3.1^{b}	1^{b}	1^{b}	1^{b}	0.8^{b}	0.75^{b}	0.72^{b}
Phenol	4.2^{b}	4.2^{b}	4.1^{b}	1.2^{b}	1.2^{b}	1.1^{b}	1.1^{b}	0.9^{b}	0.9^{b}
QACs	5^{b}	5^{b}	5^{b}	1.4^{b}	1.4^{b}	1.5^{b}	1.1^{b}	1.2^{a}	1.1^{a}
Halamid	4^{b}	4.8^{b}	4.7^{b}	0.9^{b}	1^{b}	0.9^{b}	0.9^{b}	0.8^{b}	0.8^{b}
Virkon-S	3.5^{b}	3.4^{b}	3.2^{b}	1.1^{b}	1.1^{b}	1.1^{b}	0.7^{b}	0.6^{b}	0.55^{b}
Micro Sept M	3.2^{b}	3.1^{b}	3.1^{b}	0.9^{b}	0.9^{b}	0.89^{b}	0.6^{b}	0.6^{b}	0.58^{b}

a-b Different superscripts mean significantly different column values ($P<0.05$).

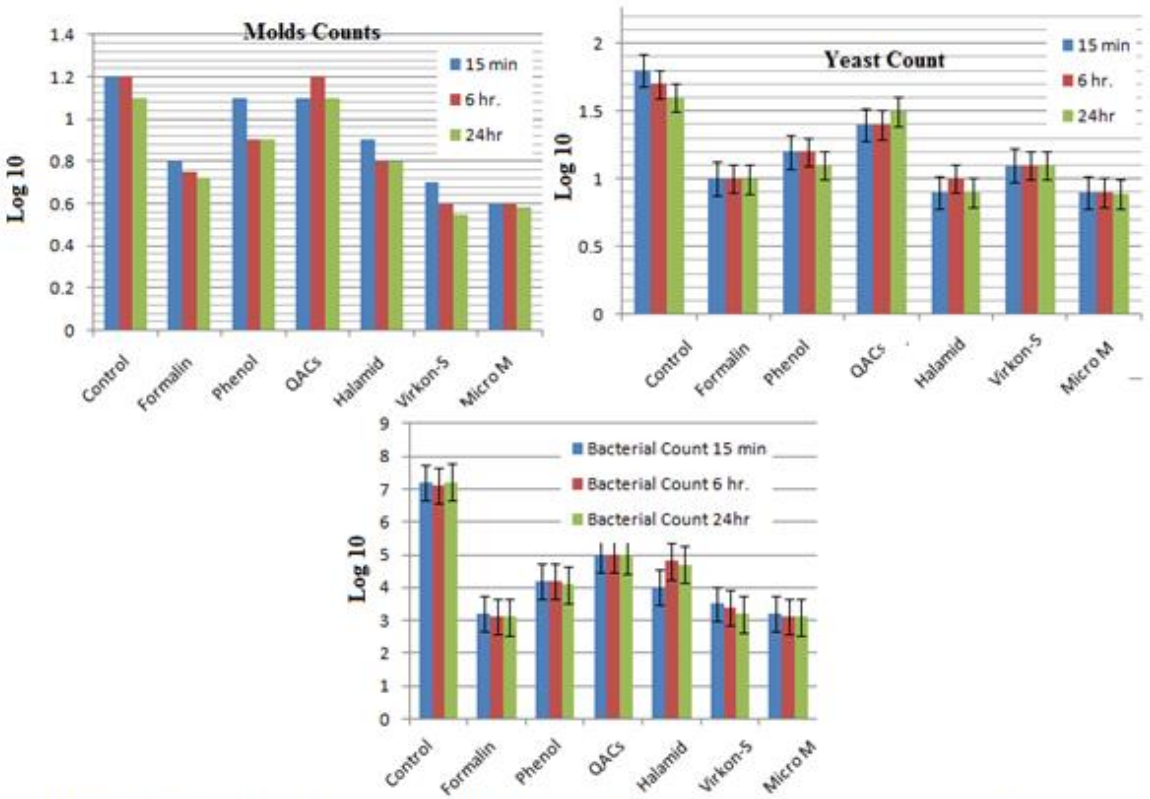

Fig. 3 : The effect of fogging application and exposure time on total aerobic bacteria, yeast and mold of poultry floor.

Table 5: The impact of disinfectants contact period (15 min) using low, high application level and cold fogging:

Method \ MIC	Bacterial Count			Yeast Count			Mold Count		
	Low	High	Fog	Low	High	fog	Low	High	fog
Control	7.2^{a}	7.2^{a}	7.2^{a}	1.8^{a}	1.8^{a}	1.8^{a}	1.2^{a}	1.2^{a}	1.2^{a}
Formalin	5.2^{b}	4.2^{b}	3.2^{b}	1.2^{b}	0.9^{b}	1^{b}	0.8^{b}	0.6^{b}	0.8^{b}
Phenol	6.2^{b}	5.2^{b}	4.2^{b}	1.4^{b}	1.2^{b}	1.2^{b}	1.1^{b}	0.7^{b}	1.1^{b}
QACs	7^{b}	6.2^{b}	5^{b}	1.6^{b}	1.4^{b}	1.4^{b}	1.2^{b}	0.9^{b}	1.1^{b}
Halamid	6^{b}	5^{b}	4^{a}	1.2^{b}	1.2^{b}	0.9^{b}	0.9^{b}	0.9^{b}	0.9^{b}
Virkon'S	5.5^{b}	4.5^{b}	3.5^{b}	1.6^{b}	1.2^{b}	1.1^{b}	0.7^{b}	0.7^{b}	0.7^{b}
Microsept M	6.2^{b}	4.2^{b}	3.2^{b}	1.4^{b}	1.2^{b}	0.9^{b}	0.9^{b}	0.9^{b}	0.6^{b}

a-b Different superscripts mean significantly different column values ($P<0.05$).

DISCUSSION

In the field trial, there was a significant ($P < 0.05$) change in aerobic bacterial count was a decrease from 6 to 24 h of exposure time. On the other hand, exposure time had no significant impact on yeast and mold counts, but did significantly increase yeast and mold populations at both the 6- and 24-h exposure times as compared with the 15-min exposure time ($P < 0.05$)/ **(Hernandez and Martinez, 2018).**

Disinfectants impacted but did not affect significantly on aerobic bacteria, yeast and mold populations at the low application rates, respectively ($P < 0.05$) except in case of Formalin and Virkon'S, where the value of decrease in log 10 was 2 and 1.7, respectively (in comparison to control (log 10 = 7.2) (Table1 and Fig.1).

Fogging of the disinfectants resulted within the greatest effect in aerobic bacteria, yeast and mold populations (Table 4 and Fig.4). The Log 10 of total aerobic populations after15 mint exposure were 3.2, 4.2, 5, 4, 3.5 and 2.2 of Formalin, Phenol, QACs, Halamid, Virkon-S and Micro-Sept M, respectively as compared to control (log 10= 7.2).

Concerning of log reduction, it had been noticed that, 3.24, 3, 2.2, 3.2 and 4.24, respectively as shown in Table 5). At the identical time, Fogging was resulted within the greatest effect in yeast and mold populations (Table 4). The Log 10 of total yeast populations after 1 5 mint exposure were 1 ,1.2,1.4,0.9,1.1 and 0.9 of Formalin, Phenol, QAC, Halamid, Virkon-S and Micro-Sept M, respectively as compared to manage (log 10= 1.8). Concerning of log reduction, it had been noticed that, 0.8, 0. 6, 0.2, 0.9, 0.7 and 0.9, respectively (Table 5).

Concerning, mold: Fogging resulted in significant effect (Table 5). The Log 10 of total mold populations after 5 mint exposure were 0.8, 1.1, 1.1.0.9, 0.7 and 0.6 of Formalin, Phenol, QACs, Halamid, Virkon-S and Micro- Sept M, respectively compared to regulate (log 10= 1.2) (Table 5). Concerning of log reduction, it absolutely was noticed that, 0.8, 0. 6, 0.2, 0.9, 0.7, and 0.9, respectively (Table 5).

Formaldehyde is bactericidal, sporicidal, virucidal, fungicides and is a monoaldehyde that exists as a freely water-soluble gas, but it works more slowly than glutaraldehyde **(McDonell and Russell, 1999).** Formalin in gaseous form was bactericidal and sporicidal. It was used very effectively with potassium permanganate in ratio of 3: 2 and it was also effective even in presence of animate or organic matter. (**Mandel, *et al.*, 2005**).

The disinfectant containing the phenolic compounds resulted within the greatest reduction in total aerobic bacteria, yeast and mold populations (0.21 log reduction) whereas the Micro-Sept M and potassium peroxymonosulfate treatment also demonstrated a big reduction in populations (0.17 log reduction). In poultry houses originate in bird droppings, including *Enterobacteriaceae* and other

bacteria with zoonotic capacity **(Cook *et al.*, 2012; Fries *et al.*, 2005).** Airborne microbial populations and aerosol production has been examined in broiler houses, hatcheries and egg processing facilities **(Clark *et al.*, 1983; Sotohy, 1989; Whyte *et al.*, 2001; Northcutt *et al.*, 2004; Karwowska, 2005; Byomi and Trabees, 2006; Duan *et al.*, 2008).**

Disinfectants without evaluation and validation during using them in farms may lead to the gradual increase in the resistance and reduction in suceptibility of the microorganisms to the disinfectants and even resistance to antibiotics **(McDonnell and Russell, 2011).** So, evaluation the potency of the disinfectants' must be put in priority to select the adequate disinfectant to reduce the pathogenic and microbial load before breeding of the birds. **Stringfellow *et al.*, (2009) and McDonnell and Russell (2011)** observed that, the only sanitation protocol which applied in poultry farms is the spraying of the disinfectants without regular evaluation and validation of the used disinfectants while the powerful of the disinfectants is implicated by the amount of organic load, formulation, humidity, temperature, pH dilution rate and salts of water, and other **factors (Islam *et al.*,2007; Stringfellow *et al.*,2009).** Thus, poor sanitation might be useless in disease control and reduce bird performance.

With the applying of ultrasonic atomization technology, liquids will be atomized into an aerosol state to get uniformly dispersed 2–4 μm droplets. The atomized droplets play the role of "seed particles" and form an aggregation nucleus within the aerosol that may effectively strike the encircling fine particles to boost the aggregation efficiency. After the disinfectant is atomized, an outsized amount of vapour is emitted within the air to extend the indoor ratio, improve the disinfectant's penetration ability on the bacterial wall, increase disinfection impact, and shorten disinfection time **(Muoz *et al.*, 2017).**

The chemicals utilized in this sort of machine are more concentrated than the chemicals employed in other spraying equipment, which also increases the killing efficiency. Other advantages of ULV misting machines includes lower risks of injury thanks to the fog cloud being nearly invisible, low volumes of carrier chemicals, lower application cost and low noise levels.

CONCLUSION

It is critical to recall that all surface wastes and loose living matter were removed prior to disinfectant treatment in the field testing. Method and rate of application are critical for an efficient disinfection procedure, as well as total pathogen destruction or even pathogen reduction to assure infection prevention. Ultra foggers are machines used huge quantities of air at decreased pressures to change solution into Nano-droplets that are distributed in the atmosphere. This type of Ultra fog machine can create tiny droplets with diameters 1 to 150 μm. Thus, poor sanitation might be useless in disease control and reduce bird performance.

Declaration of Conflicting Interests:

The authors declare that they have no conflict of interest.

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