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## Evaluation of the Viricidal Efficacy of Commercially used Disinfectants against Newcastle Disease virus

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The aim of this study was to evaluate the viricidal action of fourteen commonly used and commercially available disinfectants on poultry farms and are belonging to different groups of biocides against Velogenic Newcastle disease virus (NDV). Evaluation was carried on two different surfaces (cement and rubber) contaminated experimentally with Newcastle disease virus. Reliable disinfectants should pass the test if at least 2.8 log reductions were achieved, and no recoverable viruses were isolated after the treatment, the last point is very important in the evaluation as although the direct reduction in virus titer is critically needed in terminal disinfection, the recovered virus after disinfection or shed virus after vaccination will be always capable of introducing infections. Our results revealed that three disinfectants out of fourteen were able to achieve the previous test criteria 1. Calcium hypochlorite (5.5 log reduction on cement coupons, and 4.38 on rubber coupons) 2. Halamid<sup>®</sup> (5.5 log reduction on cement coupons, and 4.38 on rubber coupons) and 3. ZixVirox<sup>®</sup> (5.5 log reduction on cement coupons, and 4.38 on rubber coupons) while Virkon S<sup>®</sup>, PIQuat 20<sup>®</sup>, Synergize<sup>®</sup>+Formalin, and GroundZero<sup>®</sup> had only achieved the required log reduction on both surfaces but failed to stop viral propagation, it's also worth to mention that disinfectants Aquazix E52<sup>®</sup> and FumagriEffisafe<sup>®</sup> achieved the required log reduction on cement surface only but also failed to stop viral replication, disinfectants Synergize<sup>®</sup>, Formic, and Citric acid neither achieved the required log reduction on both surfaces, nor stopped the virus propagation after treatment.

**Keywords:** Viruses; Biocides; Newcastle disease virus; Efficient; Evaluation.

### INTRODUCTION

Poultry farming is one of the rapidly developing sectors plays an important role in the Egyptian food security. The sustainability of this sector is continuously threatened by emergence of different viral pathogens as Newcastle disease

virus and avian influenza virus. Newcastle disease virus (NDV) was first identified in Egypt at 1948 (Daubney and Mansi 1948), and since the disease became widely spread throughout the country and recorded as an endemic by the beginning of the 1960 (El-Nassary and Eskarous, 1960).

Trials for controlling the disease were practiced by mass vaccination programs using combined live attenuated and inactivated vaccines to develop solid immunity against the virus. (Alexander, 1998, Ewies et al., 2017). From the early months of 2011, many NDV outbreaks with elevated mortality rates were recorded regardless the adopted intensive vaccination programs, these outbreaks were attributed to the circulation of the new genotype VII d that wasn't reported before in Egypt. (Radwan et al., 2013, Hussein et al., 2014, Oraby et al., 2017 and Ewies et al., 2017).

Viruses differ in their resistance to chemical disinfectants, regarding the early classification published by Noll and Youngner 1959 viruses were classified into three groups (A, B, and C) according to their resistance to chemical biocides this classification depended on the virus size (large, moderate and small) and the presence or absence of lipids envelope around the virus, these criteria will mostly influence the resistance to chemical agents. This conclusion had been also confirmed by many authors (Klein and Deforest, 1965, 1983; Evans et al., 1977; Scott, 1979; Maris, 1986, 1990). Newcastle disease virus (NDV) belongs to category A, which includes all enveloped viruses of intermediate to large size which make the virus susceptible to the most of categories of disinfectants (Ausvetplan, 2011).

Regardless the previous fact, in the field conditions The efficacy of chemical disinfectants can be severely influenced by many factors such as contact time, temperature, dryness, properties of the material (porous vs. non-porous) and organic load (e.g. manure, dust) which can dilute and inactivate the disinfectant or sheltering micro-organism, so evaluation of disinfectants against the virus must take in consideration this critical factors (Jang,Y et al.,2014).

Disinfectants which are theoretically active against category A viruses can be grouped into detergents, organic acids, oxidizing agents (chlorine and chlorine releasing agents-oxygen releasing agents), aldehydes (Formaldehyde and glutaraldehyde), phenolic compounds (black fluids, white fluids, and coal tar derivatives) and surfactants (Ausvetplan, 2011).

The goal of our study was to evaluate the general viricidal ability of fourteen commercially available disinfectants in the Egyptian markets individually against velogenic Newcastle disease virus Genotype VII on different surfaces (cement and rubber) found in the Egyptian poultry farm environment.

## MATERIALS AND METHODS

### Tested Virus:

NDV Genotype VII was isolated from Giza governorate – Egypt (Gene bank accession number KF709445). The virus was isolated from the tracheas and dropping of 60 days old Bovans Brown pullets suffering from high mortalities, the flock was vaccinated with Inactivated NDV at 21 days and Live Lasota virus at 27 days. Virus stock was titrated and virus EID<sub>50</sub> was calculated using the method of Reed and Muench (1938).The purpose of using such locally isolated virus strain was to give the test the maximum simulation of the Egyptian field conditions.

### Chemical disinfectants, Neutralizers, Building surfaces and Interfering reagents

The used Disinfectants in this study (Table 1) were tested individually according to Guidelines of EPA 2005 for effectiveness against NDV for one minute and neutralizers were used to remove any residual disinfectants (Table 2). For evaluation of the viricidal ability of each disinfectant; two types of surface coupons were used (fig 1); cement which resembles to poultry house floors, walls and roofs (manufactured in Arab Contractors Company in Egypt with dimensions 2 x 2x 1cm<sup>3</sup>) and rubber that resembles to shoes usually used by poultry workers in poultry farms (was cut from shoes with dimensions 2 x 2x 1cm<sup>3</sup>).

### Two different interfering factors were used:

A. Water hardness: The tested disinfectants were diluted using 300 ppm hard water solution freshly prepared on the day of use. The hard water solution was prepared according to (Bloder,2009)

B. Organic challenge: 5% organic matter solution was prepared by adding by adding 3g of yeast extract and 2 grams fetal bovine serum to 100ml bi- distilled water.

### Viricidal efficacy assays:

According to the EPA, 2005. Guidelines; the disinfectant evaluation study should have three groups (test, positive control and cytotoxicity).

A-For test group, three coupons from each surface (cement and rubber) were sterilized by autoclaving and placed in sterile Petri dish. Each coupon was coated with 0.2 ml of infective allantoic fluid and 0.2 ml of 5% organic matter

solution and left to dry about 1 hour at room temperature.

**Table (1): The used Disinfectants:**

Disinfectant	Composition	Manufacture Dilution	Manufacture
Virkon S	Potassiumperoxymonopersulphate (KMP) 55% Sodium chloride 3%	5 g / L	DuPont USA
Synergize	Glutaraldehyde 7%, Benzalkonium chloride 26%	5 Cm / L	NEOGEN USA
AQUAZIX	HYDROGEN PEROXIDE 50%	30 Cm / L	BBZIX SPAIN
ZIXVIROX	PERACETIC ACID 5% Hydrogen peroxide 25 % Acetic Acid 6.5 %	10 Cm / L	BBZIX SPAIN
HALAMIDE	CHLORAMINE T 100%	10 Cm L	Axcentive FRANCE
Ground zero	Glutaraldehyde 3.5%, Iodophor 3%	5 Cm / L	NEOGEN USA
IODIS	1.75 % Titratable iodine	10 Cm / L	NEOGEN USA
Ca. Hypochlorite	Ca. Hypochlorite 70 % available Chlorine	750 PPM	SIGMA-ALDRICH
Dyne-o-might	IODOPHOR 0.48%+ PROPIONIC ACID 20 %	50 Cm/L	NEOGEN USA
Formic acid	FORMIC ACID 99%	10 g / L	EL-NASR EGYPT
Citric acid	CITRIC ACID 99%	10 g / L	EL-NASR Egypt
PIQUAT 20	3 <sup>rd</sup> Generation QACS 20 %	5 Cm / l	NEOGEN USA
Fumagri EFFISAFE	Orthophnylphenol 15 % Chlorocresol 5 %	5 Cm / l	LCB FRANCE
SYNERGIZE + FORMALDHYDE	Glutaraldehyde 7% Benzalkonium chloride 26 % Formalin 40%	5cm / l	NEOGEN USA

**Table (2): The used Neutralizers:**

Neutralizer	Disinfectant	Reference
Sodium thiosulphate 1%	Calcium hypochlorite Halamide Citric acid Formic acid	Russell et al., (1979)
Sodium thiosulphate 0.5%	VIRKON S®	BS EN 1656:2000
Sodium thiosulphate 1% - sodium polysorbate 1% (Tween 80) – sodium bisulphate 1% - Lecithin1% - 1% sodium thioglycolate	SYNERGIZE Formaline FumagriEffisafe PIQUAT 20	Dey and Engley (1994)
Sodium thiosulphate 1% - sodium polysorbate (Tween 80) 1% – sodium bisulphate 1%	ZIXVIROX 5%® AQUAZIX	Espigares et al., (2003)



**A. Cement coupons**



**B. Rubber coupons**

**Figure 1. The used surface 1. Cement surface – 2. Rubber surface**

Coupons were covered with 3 ml of the tested disinfectant (Table 1), which kept on coupons for one minute then each coupon was scraped by sterile pipet, the fluid was aspirated from the Petri dish and jetted back onto the coupon three times to dislodge virus from the coupon. The fluids from the Petri dish were pooled into a single tube; 1 ml from the pooled fluid was added to 9 ml neutralizer (specific for each disinfectant) to stop the action of disinfectants, then 10-fold serial dilutions ( $10^{-1}$  to  $10^{-3}$ ) were prepared in sterile PBS.

B- For control group, the pooled fluid from the coupons of positive controls was diluted 10-fold serial dilutions ( $10^{-1}$  to  $10^{-6}$ ). While the cytotoxicity group was diluted once ( $10^{-1}$ ) and inoculated into three SPF ECEs

C-The third group assess the cytotoxicity of the disinfectant/neutralizer mixture. Virus re-isolation attempts were carried out using each dilution by inoculation via allantoic route of 9-11day old specific pathogen free (SPF) embryonated chicken eggs (ECEs). Eggs were candled daily for 3 days and the dead eggs were chilled at 4°C for 24 h, then the harvested allantoic fluids were tested for HA activity and EID<sub>50</sub> was calculated using the method of read and munch 1948

#### Calculation of Neutralizing index (NI):

Neutralization index (NI): is a Mathematical numerical method used to express the ability of a disinfectant to inactivate viruses. The NI is calculated using the following equation  $NI = \frac{Pc}{Ta}$  Where PC is the titer of the positive control plate and Ta is titer of the recovered virus from the disinfectant-treated plates when no recoverable viruses were isolated from the disinfectant treated plates NI was considered equal to a titer of 1.2. (Lombardi et al., 2008).

#### 5. Test judgment (Pass or Fail criteria):

Disinfectant will pass the test if it could achieve a reduction in the virus titer equals to or more than 2.8 NI, while the positive control titer must be 4 or more, and no recoverable virus from any treated coupon was isolated.

## RESULTS AND DISCUSSION

The choice of efficient disinfectant is critical in establishing a successful sanitation program as not all disinfectants are effective against the major pathogens that cause economic Loss diseases in the poultry sectors. So, laboratory evaluation of the disinfectants should always consider some critical factors within the environment at which disinfectant will act like; surface type, water

hardness, organic matter and contact time that might directly impact on the biocidal ability of the disinfectant (McDonnell and Russell, 1999). In the present study, we tried to give our test the maximum simulation of the Egyptian field conditions by using locally isolated Egyptian velogenic NDV strain that causes high mortality problems in the Egyptian poultry farms, and using two different surfaces (cement and rubber), the first one was manufactured from cement resemble to the Egyptian poultry house floor, and the second surface was made up from shoes rubber to mimic the shoes sole which may bring viral infection to the poultry house.

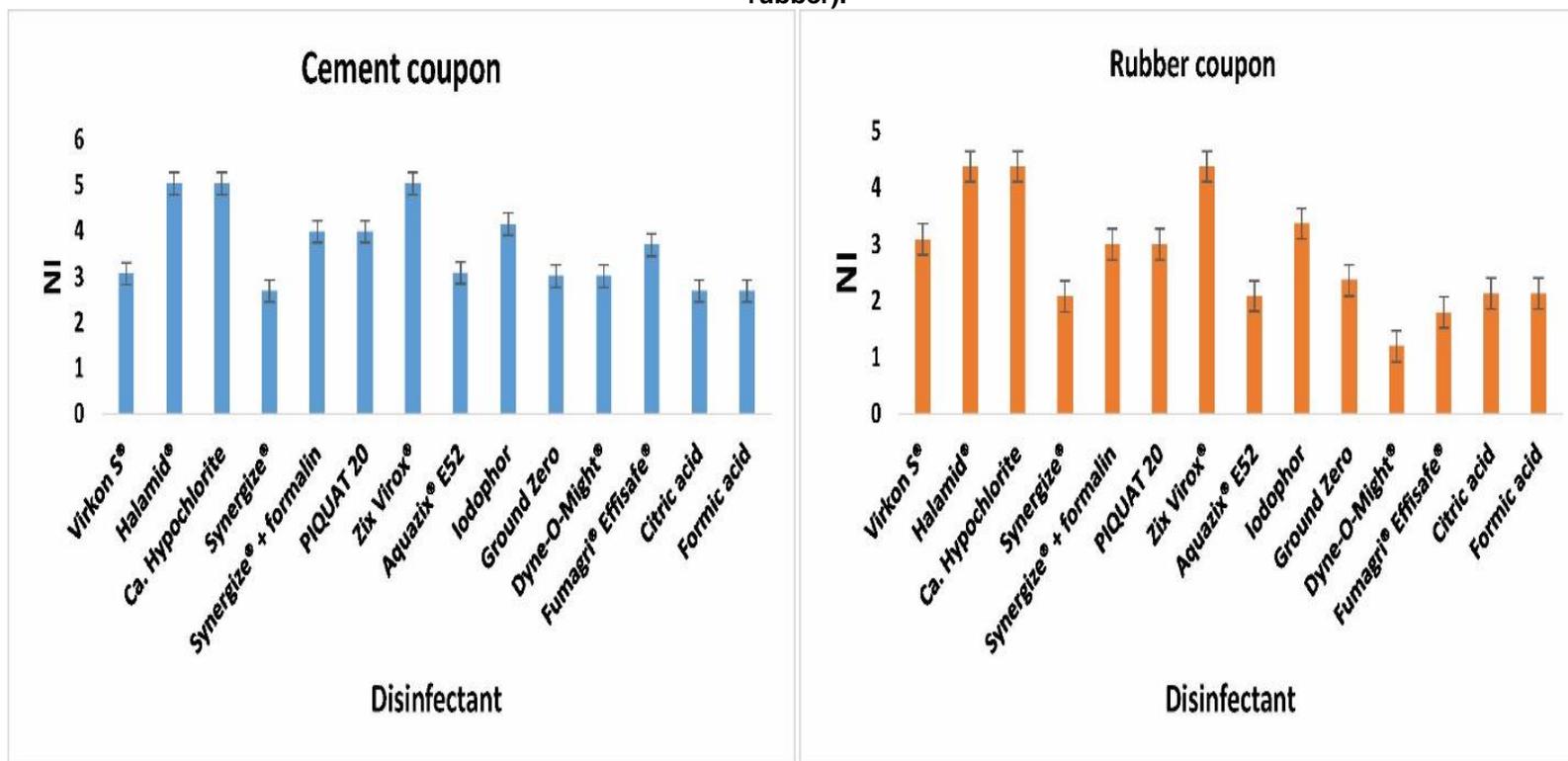
According to the United States Environmental Protection Agency guidelines (EPA, 2005), every disinfectant must be tested for each individual organism to be involved in the control of that organism., disinfectant will pass the evaluation test if it achieved a reduction in the virus titer from 2.8 to 4 log<sup>10</sup> reductions, and no recoverable virus from any treated coupon was detected. No recoverable virus after using the disinfectants is very important criterion in the field conditions as recoverable viruses may still be able to induce infection to poultry flocks. Likewise, we also had chosen a short contact time (one minute) (Patnayak et al., 2008) as our field experience showed that the rapid action of the disinfectant is very important in Egyptian field as workers are always reluctant to spent more than one minute to disinfect their shoes in the foot dip, beside the inaccurate calculation of water amounts and disinfectant concentration that needed to terminally disinfect the poultry house which affects the efficacy of the terminal disinfection process.

By taking in consideration the previous judgment criteria, although most of disinfectants had achieved the required Log reduction (2.8 or more) only three of them were successful in preventing viral particles from propagation in the embryonated chicken eggs (ECE) after treatment on the two used surfaces. By giving a quick look to the three successful disinfectants we will find that they are all oxidizing agents (Calcium hypochlorite (0.7g/l) in 5.5 log reduction on cement coupons, and 4.38 on rubber coupons – Halamid® (1%) 5.5 log reduction on cement coupons, and 4.38 on rubber coupons – Zixvirox® (1%) 5.5 log reduction on cement coupons, and 4.38 on rubber coupons) (Table 3 and Fig 2).

**Table (3): Viricidal efficacy of fourteen disinfectants and their compositing used on two different surfaces against Newcastle disease virus**

Disinfectant group	Cement coupons (NI)	Recoverable virus (Yes / No) Pass / Fail	Rubber coupons (NI)	Recoverable virus (Yes / No) Pass / Fail
<b>1. Chlorine releasing agents:</b>				
A. Virkon S	3.08	Yes – Fail	3.09	Yes – Fail
B. Halamid	5.05	No – Pass	4.38	No – Pass
C. Ca. Hypochlorite	5.05	No – Pass	4.38	No – Pass
<b>2. Aldehyde and Quaternary ammonium compounds:</b>				
A. Synergize	2.7	Yes – Fail	2.08	Yes – Fail
B. Synergize + formalin	4	Yes – Fail	3	Yes – Fail
C. PIQUAT 20	4	Yes – Fail	3	Yes – Fail
<b>3. Oxidizing agents</b>				
A. ZIXVIROX	5.05	No – Pass	4.38	No – Pass
B. AQUAZIX E52	3.09	Yes – Fail	2.08	Yes – Fail
<b>4. Iodine related compounds:</b>				
A. Iodophor	2.09	Yes – Fail	1.88	Yes – Fail
B. GROUNDZERO	3.18	Yes – Fail	3.92	Yes – Fail
C. DYNEOMIGHT	3.02	Yes – Fail	4.05	No – Pass
<b>5. Phenolic compound</b>				
A. FumagriEffisafe	3.7	Yes – Fail	1.8	Yes – Fail
<b>6. Organic acids</b>				
A. Citric acid	2.7	Yes – Fail	2.13	Yes – Fail
B. Formic acid	2.7	Yes – Fail	2.13	Yes – Fail

Figure 1. Neutralization index of fourteen disinfectants evaluation against Newcastle disease virus on different surfaces (cement and rubber).



The first two disinfectants were Chlorine releasing agents (calcium hypochlorite – chloramines) this result came in agreement with results obtained by (Patnayak et al., 2008) how evaluated a sodium hypochlorite disinfectant against NDV using stainless still disks, the disinfectant was able to achieve 3 log reductions after 1 min exposure time.

The third disinfectant was a peracetic acid-based disinfectant, our results disagreed with results obtained by (Patnayak et al., 2008) how evaluated a peracetic acid-based disinfectant that could inactivate NDV in a surface test model after 5 min while the disinfectant failed to achieve 3 log reductions after one min despite not using any interfering materials in that test.

Both results disagreed with results recorded by (Kassaify et al., 2007) how used Peracetic acid in suspension test model with 15 % skimmed milk as interfering material against NDV for 30 min and the disinfectant failed to inactivate the virus. This difference in results may be attributed to the original concentration of the used PAA; also the high concentration of the interfering material that used in this study may have played a role in the inactivation of the PAA, which will highlight the importance of the cleaning process before applying PAA as a disinfectant against NDV

Unfortunately, the viricidal activity of hydrogen peroxide have not been widely studied, in this study stabilized hydrogen peroxide based disinfectant Aquazix® E52 (3%) failed to stop viral propagation on both surfaces but succeeded to achieve the required log reduction on cement surface only (3.09 log reduction on cement coupons, and 2.08 on rubber coupons) (Table 3 and Fig 2) this may be attributed to the surface nature and microtopography. Our results came in

agreement with (Wanaratana, S. et al., 2010) as he also failed to inactivate H5N1 avian influenza virus (class A virus) using Liquid hydrogen peroxide.

Virkon S® is a Potassium momopersulphate , organic acids (malic acid) , inorganic acids (sulphamic) and halide salt (sodium chloride) based system that react to produce hypochlorous acid which is known to be a potent viricide, in our results virkon S® (0.5%) failed to achieve complete inactivation of the viral particles on both surfaces (3.08 log reduction on cement , and 3.09 on rubber coupons) (Table 3 and Fig 2) although it could reach the required log reduction this finding disagreed with results obtained by (Patnayak et

al., 2008) study in which virkon S® was able to inactivate the virus after 1 min exposure , but he didn't mentioned if there were recoverable viruses after the treatment or not, the difference between the two findings can be also attributed to the lack of interfering materials in (Patnayak et al., 2008) study as interfering materials are known to inactivate disinfectants, (Xiuying, et al., 2001) also inactivated NDV after 5 min and 10 mins using suspension test but no interfering materials were used.

In our study, the used glutaraldehyde and QACS based disinfectant (Synergize®) 0.5% did not success in either complete inactivation of the NDV (2.7 log reduction on cement coupons, and 2.08 on rubber coupons) (Table 3 and Fig 2) nor achieving the required log reduction after 1 min in both surfaces. This finding agreed with results obtained by (Patnayak et al., 2008) who evaluated glutaraldehyde-based disinfectant 0.5% that was not capable of inactivating the virus after 1 min but could inactivate it after 3 mins. Also, (Kassaify et al., 2007) used glutaraldehyde and QACS based disinfectant that failed to inactivate the virus after 30 mins using 0.5% and 1% concentrations. Formalin was extensively used for disinfection of poultry house in Egypt, in our study we had combined formalin and with previously mentioned glutaraldehyde and QACS based preparation (Table 3 and Fig 2) to potentiate its action and used at 0.5% dilution rate but surprisingly the results did not changed but it worth mentioning that addition of formalin made the formula reach the required log reduction on both surfaces (4 log reduction on cement coupons , and 3 on rubber coupons) this findings was in agreement with results obtained by (Qayyum et al., 1999) who evaluated glutaraldehyde, formalin and QACS based disinfectants against the NDV and failed to achieve complete inactivation after 30 mins using 0.5 % concentration but succeed after 15 mins using 1% concentration.

QACS are known for their ability to inactivate enveloped viruses, in our study although the used QACS preparation PI Quat 20® (0.5 %) could achieve the required log reduction on both surfaces (4 log reduction on cement coupons , and 3 on rubber coupons) (Table 3 and Fig 2) but it was not able to prevent the virus replication on ECE this findings were similar to the findings reported by (Patnayak et al., 2008) how evaluated three similar Alkyl dimethyl benzyl ammonium chloride based disinfectants with different results one of them arrived the required log reduction after 1 mins while the reaming two disinfectants

failed to inactivate the virus, our finding came in agreement to somewhat with findings obtained (Kassaify et al., 2007) who evaluated QACS based disinfectant against NDV and failed to inactivate it after 30 mins. (Abe et al., 2007) reported in their study that they were able to inactivate the NDV after 30 mins using 0.1 % and 0.2% Benzalkonium chloride in a suspension test model but no interfering material or neutralization method were mentioned this finding come in contrast with the finding reported by (Qayyum et al., 1999) as he failed to inactivate the NDV the virus after 45 min using 0.1% concentration but could reach the inactivation level after 30 mins using 0.5 % concentration, although they used the same test conditions (No interfering materials or neutralization method were mentioned in that study).

Iodophors are known in the Egyptian markets as viricidal agents, beside poultry farm owners always prefer to use iodophors when facing viral outbreaks. In our study, three iodophors based disinfectants (Iodis<sup>®</sup>, Ground Zero<sup>®</sup> and Dyne-O-Might<sup>®</sup>) (Table 3 and Fig 2) were evaluated for their viricidal activity. The results were unexpected as the disinfectant based only on iodophor Iodis<sup>®</sup> (1%) had failed to achieve the required log reduction on both surfaces (2.09 log reduction on cement coupons, and 1.88 on rubber coupons), and to prevent the viral propagation in ECEs. Likewise, GroundZero<sup>®</sup> (0.5%) preparation at which glutaraldehyde is being added, had failed in preventing the viral propagation but had achieved the required log reduction (3.18 log reduction on cement coupons, and 3.92 on rubber coupons), while Dyne-O-Might<sup>®</sup> (5%) disinfectant which based on iodophor and propionic acid succeeded to reach the required log reduction on both surfaces (3.18 log reduction on cement coupons, and 4.05 on rubber coupons), another interesting finding that Dyne-O-Might<sup>®</sup> inhibit the viral propagation on the rubber surface but not on the cement surface, this may be attributed to the surface nature, microtopography (Lombardi et al., 2008). These findings support the hypothesis that adding a compatible material to the iodophor will enhance their viricidal activity against NDV. Our results also came in agreement with results obtained from (Kassaify et al., 2007 and Qayyum et al., 1999) as in both studies iodophor disinfectants failed to achieve complete inactivation of NDV after 30 min, 45 min respectively.

In this study, phenolic based disinfectant failed to achieve the required log reduction on

rubber coupons but not on cement coupon

Synthetic phenol based disinfectant were heavily studied for their efficacy against NDV, In our study, phenolic based disinfectant FumagriEffisafe<sup>®</sup> (0.5%) failed to achieve the required log reduction on rubber coupons but not on cement coupon (3.7 log reduction on cement coupons, and 1.8 on rubber coupons) (Table 3 and Fig 2), this result to somehow came in agreement with the findings obtained by (Patnayak et al., 2008) how completely inactivated the virus using chlorphenol based disinfectant after 1 min but failed to inactivate it after 1 min when used orthophenylphenol based disinfectant after 10 mins. (Qayyum et al., 1999) could inactivate the virus using 0.4% and 0.6% respectively after 15 mins.

Organic acids as Formic and Citric acid (1%) were evaluated in our study, none of the two acids were able to pass the test criteria, they neither achieve the required log reduction nor preventing the viral propagation (2.7 log reduction on cement coupons, and 2.13 on rubber coupons) (Table 3 and Fig 2), unfortunately no available literature is available concerning the evaluation of organic acid against NDV but literature were available on Low pathogenic avian influenza virus (Enveloped – class A virus), (Lombardi et al., 2008) evaluated 1 % citric acid in surface test model against H7N2 virus the disinfectant was able to completely inactivate the virus in different surfaces ( metal – wood – plastic), we also had reached the same conclusion in previous research, as we evaluated formic and citric acid with the same concentration against two H5N1 highly pathogenic avian influenza virus on cement coupons and found that none of them were able to inactivate the virus after 5 or 30 minutes (Gamal et al., 2013).

## CONCLUSION

In conclusion, halogen compounds (Halamid<sup>®</sup> and Calcium Hypochlorite powder) and oxidizing agents (Zix Virox<sup>®</sup>) showed the best biocide activity for the disinfection. The information on the lowest efficient concentration and the shortest contact time of these three disinfectants will be useful during selection of the most appropriate biocide against NDV.

## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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#### AUTHOR CONTRIBUTIONS

A.M. Gamal performed the experiments and wrote the manuscript, S.T. Moubark, M. M Aly, H. M. Elagrab, T. F. Ismail and O.K. Zahran designed the experiment and reviewed the manuscript. All authors read and approved the final version.

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