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Evaluation the efficacy of some types of disinfectants (Traditional and Nano types) against Brucella melitensis.

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Abstract

Brucellosis is a zoonotic disease that transmitted by many ways between animals and humans. Disinfection of environments surrounded animals and good removal of infection from animal house has a very important role in the prevention and control of brucellosis. In our study we used some types of disinfectants against Brucella melitensis to evaluate its efficacy and if it is effective against Brucella melitensis in different environmental conditions. Our study included some traditional types of disinfectants and antiseptics (Virkon® S, Cidex, Sodium hypochlorite, Betadine and Dettol) and three types of Nano-disinfectants (Dettol with Silver-NPs, Glutaraldehyde with silver-NPs and Calcium oxide-NPs). Reduction rate was used for estimation the efficacy of different types of used disinfectants. The results showed that the bactericidal effects of the used disinfectants were influenced by increasing of their concentration and more exposure time specially Vircon S, however presences of dirty conditions and low temperature significantly decrease the efficacy of disinfectants specially Dettol. In other side Nano-disinfectants had better effect than ordinal types specially Glutaraldehyde with silver NPs. Our study suggested that Brucella melitensis is affected by commonly used disinfectants. However, the bactericidal efficacy was decreased with presence of dirty conditions and low temperatures. Nano-disinfectants had superior effect on Brucella.

Key words: Nano, disinfectants, traditional, Brucella.

Introduction:

Brucella spp. is a Gram-negative bacterium spread widely among different hosts through many means of transmission (**Corbel 2006).** *Brucella* infection causes abortion of pregnant animals at late stage of gestation also causes orchitis in males in both animals and human **(Alton *et al.*, 1988).** *Brucella* microbe is discharged in milk, discharges from uterus or vagina after abortion or parturition, fetal membranes and into urine of infected animals. Moreover,

Brucella can stay alive in environment for long time, that depending on environmental conditions such as low temperature, pH, and humidity (**Al-Majali *et al*., 2009).**

Brucella could survive in many materials as dust, drinking water or manure and slurry. Also aborted fetuses, soil, meat and dairy products may keep the microbe inside it for considerable periods of time depending on suitable condition **(WHO, 2006).** Infection with brucellosis occur due to direct or indirect contact with infected animals or contaminated environment **(Foster *et al*., 2007).** *Brucella* although it can remain alive in dirty environment but it is known to be susceptible to heat treatment, disinfection, and direct sunlight **(Pappas *et al*., 2005).**

Disinfection is a very important element of brucellosis control program as well as other efforts so choice of the type of disinfectant should be after good evaluation **(OIE, 2004).** Each used disinfectant has advantages, side effects and suitable application method. For example, Glutaraldehyde is very strong disinfectant, it used for disinfection of metals and material which is sensitive to heat but it is very corrosive to skin. Chlorine is an intermediate level disinfectant that used for disinfection of biological material, equipment, medical supplies and environmental surface. It is of low cost, fast acting, but it has corrosive effect on metals and irritant to skin **(Rutala, 1996).** Many researches indicated that *Brucella spp.* is sensitive to most available disinfectants as halogens, ethanol, phenol and formaldehyde but every type needs to be evaluated to decide the proper mean of application **(Corbel, 2006).**

Nano-based disinfectant can be used to reduce the bacterial burden in environment and can be effective against resistant organisms as *E-coli*, *salmonella spp*. and *Martha*, so using of new types of Nano disinfectants would be helpful for control of many types of infectious bacteria **(Saengkiettiyut *et al*., 2008; Rai *et al*., 2012).**

Silver known as a strong antibiotic and has wide range of industrial applications in healthcare and external medicine, also silver nano particles had

bactericidal effect against wide sector of bacteria and increase the efficacy of other antibacterial agents if combined with it **(Hossain *et al.*, 2014).** Silver nano particles had a good efficacy against bacteria. Its Killing effect possibly occurred due to bacteriostatic effect of silver. Although silver was so effective for killing the pathogenic bacteria, the formation of toxic product inside bacterial cells may have some irritable reaction to skin at the site of application (**Sökmen *et al.*, 2001).** Nanoparticles of Silver (Ag-NPs) represent an important nano medicine-based advance in the fight against poly-resistant bacteria. In laboratory the antibacterial activities of kanamycin, erythromycin, chloramphenicol and ampicillin were increased in the presence of Ag-NPs against tested bacterial strains, so it is recommended to adding of Ag-NPs to anti-bacterial agents to enhance its efficacy **(Fayaz *et al*., 2010).**

Inorganic nano-metal oxides as (MgO, ZnO and CaO nanoparticles) can be used as anti-microorganism agents for pathogen control. It has oxidative effect against microorganism cells. Besides, it has good penetration power and good stability under environmental condition **(Cha *et al*., 2012).**

The aim of our study is to evaluate the efficacy of different types of disinfectants against *Brucella spp*. and to compare between the traditional types of disinfectants and nano-disinfectants to estimate the suitable type to be used in brucellosis control program.

Materials and method:

Bacterial suspension of *Brucella melitensis*. (Wang *et al*., 2015).

Brucella melitensis biovar 3 is an endemic strain in Egypt. It was isolated from slaughtered serologically positive animals and its isolation and typing took place at *Brucella* department-Animal Health Research Institute, Cairo, Egypt. It was reactivated and cultured 3 days before its using. It was plated onto tryptone soya agar (TSA, Oxoid) and incubated at 37°C. A bacterial suspension at optical density (OD) 600=1.0 (equivalent to about $2\text{-}4\times10^9$ (colony forming unit) cfu /mL was diluted with physiological saline and kept until the test).

Disinfectants suspension preparation. (Park and Chen, 2011).

Five types of traditional disinfectants including Potassium peroxy monosulfate (Virkon® S), Glutaraldehyde (Cidex), Sodium hypochlorite (Bleach), Povidone iodine (Betadine) and Chloroxylenol (Dettol). Three types of Nano disinfectants including Chloroxylenol with silver-NPs, Glutaraldehyde with silver-NPs and Calcium oxide NPs. All disinfectants were freshly prepared according to the manufacturer's instructions prior to test as showed in table (1).

Determination of the Minimum Bactericidal Concentration (MBC) of different types of disinfectants. (Uzer *et al*., 2016).

Each disinfectant was diluted by a two-fold serial dilution method using sterile distilled water in test tubes, every tube have 1.9 ml of disinfectant. 100 µL of bacterial suspension (2-4×10^9cfu/mL) was added to test tubes containing the different concentrations of examined disinfectant (ten folds of manufacture concentrations), vortexed and incubated for 20 min. Sterile distilled water used as a control. After the exposure time, 100 µl of the bacterial suspension from all concentrations of each disinfectant was spread on the TSA plates. The growth was examined after incubating for 72 hours at 37°C, and the minimal inhibitory concentration (MIC) values were recorded as showed in table(2). The lowest concentration at which the bacteria could not survive was recorded as MIC. Then, 0.5 mL MIC bacterial suspensions were sub-cultured in 4.5 mL liquid media without chemicals at 37°C to detect any bacterial survival activity. And the concentration at which complete bacterial killing occurs recorded as MBC. Testing of every disinfectant dilution was performed in triplicate manner.

The bactericidal effect of disinfectants under different environmental conditions (Randall *et al*., 2004):

To evaluate the efficacy of disinfectants under different environmental conditions, we used the MBC of each disinfectant with saline, soil and fecal matter. Then, 20% suspensions of soil and fetal matter which collected from animal house and sterilized by autoclaving were prepared and stored till examination. An amount of 1.9 mL of MBC of each disinfectant was added to

each test tube then 100 µL of bacterial suspension were added to the test tubes. Then, 2 mL from saline, sterile soil and sterile fecal suspension added to each tube. Sterile distilled water was used as a negative control, after that, all tubes kept at room temperature (25°C) for different exposure time (1 min, 5 min and 10 min). Ten-fold dilution was used for every test tube and the contents plated onto TSA media to estimate the viable bacteria counts.

The bactericidal effect of chemical disinfectants at low temperatures (Suller and Russell, 1999):

To evaluate the effect of low temperatures on the bactericidal effects of each disinfectant, suspension of bacteria with MBC of the disinfectants and with saline, soil and fecal matter were prepared as described before and kept on ice (temperature of suspension less than 5°C) for 1, 5 and 15 minutes after that, incubated at 37°C for 72hrs then evaluated to determine the reduction rate.

Calculation of reduction rate of bacterial count, Olsen & Bakken (1987):

All cultured plates were incubated at 37˚C for 72 hrs. and any growth on these plates was recorded with regard to the used concentration of each chemical and contact time. The percentage of reduction was calculated with the formula as following; $REDUCTION\ PERCENT = \frac{s(100)}{APC}$

Where S = surviving bacterial (CFU ml-1) and ACP = Aerobic counting plate initial (CFU ml-1).

The disinfectant was considered as excellent effective when it demonstrated a 99.999% of bacterial reduction. The mean of the temperature during the assays was 37 ± 1.0°C.

Results:

All traditional disinfectants had good reduction rate when compared with saline and its efficacy increased with time. While with organic matters as (soil and feces) its reduction rate decreased specially Dettol and Cidex which had the lowest reduction rate with the presence of organic matters (Dettol; 75% and 73%. Cidex; 70% and 69% with soil and fecal matters respectively) as showed in Figure (1).

Nano disinfectants had advance on traditional types, as the effect of Chloroxylenol after combination of Silver NPs was (96%, 78%, and 77% with saline, soil and feces, respectively) and Glutaraldehyde after combination of Silver NPs was (99%, 90% and 84% with saline, soil and feces, respectively). While, the effect of Calcium-NPs was (90%, 70% and 75% with saline, soil and feces, respectively) as showed in Figure (2).

The reduction rate of all traditional disinfectants decreased in low temperature as it decreased moderately in presence of saline. While, with presence of organic matters it dramatically decreased specially Vircon S (70%, 50% and 49% with saline, soil and feces respectively) and Cidex (53%, 46% and 43% with saline, soil and feces respectively) which had the lowest reduction rate while other types had acceptable reduction rate as showed in Figure (3).

Nano disinfectants had good reduction rates at low temperature even with presence of organic matters especially Glutaraldehyde with silver-NPs (90%, 78% and 88% with saline, soil and feces respectively) and Dettol with silver-NPs (84%, 80% and 80% with saline, soil and feces respectively) which had the highest reduction rate as showed in Figure (4).

Discussion:

Brucellosis is very important zoonotic disease infect nearly all animals and human. Moreover, it causes many loses in animal and human sectors. *Brucella* microorganism present in secretion of infected animals and polluted the surrounded environment so good hygienic measures including strict disinfection

should be applied to reduce the prevalence of the disease. Brucellosis still endemic in Egypt especially Nile delta, it distributed between all types of domestic animals. Moreover, *Brucella* organisms was isolated from catfish of Nile **(Tittarelli *et al*., 2005; Wareth *et al*., 2014).**

The efficacy of traditional disinfectants against *Brucella melitensis*:

In our study all traditional disinfectants had a good reduction rate when applied with saline. While with organic matters as (soil and feces) its reduction rates decreased specially Dettol and Cidex which had the lowest reduction rate when applied in the presence of organic matters even for longer time periods as showed in Figure(1). Our results agree with **Park & Chen, (2011)** they reported that povidine-iodine have a good effect on *Brucella* microorganism so can be used in brucellosis control program**.** Alkaline disinfectants as (quaternary ammonium compound, sodium dichloroisocyanurate, potassium monopersulphate/sodium dichloroisocyanurate) have excellent efficacy against *Brucella spp.* even in presence of organic matters **(Yoo 2009).** Evaluation of commonly applied disinfectants and antiseptics in veterinary field against *Brucella* organisms indicated that all commonly studied disinfectants had a good efficacy, but some types need more contact time or increasing of its concentration especially with organic matters **(Adel *et al*., 2015).**

The reduction rate of all disinfectants decreased in low temperature, it slightly decreased with saline while with presence of organic matters it dramatically decreased specially in using vircon S and cidex which had lowest reduction rate while other types had higher reduction rate. these agrees with the results of **McDonnell and Russell, (1999),** they mentioned that the bactericidal action of disinfectants usually increases with the increase of contact time and increase of temperature, liquid disinfectants had less activity or be completely inactivated under dirty conditions or at cold conditions due to decreasing of its reaction or organic substances prevent the disinfectant to reach and contact with the bacterial cell**.**

Our result also agree with **Wang *et al.*, (2015)** who reported that the examination of the activity of six types of disinfectants including; quaternary ammonium compounds (QAC), aldehydes, halogens, phenol and alkaline compounds by using the MBCs of every

type. Their results indicated that all previous compounds were active against *Brucella spp.* specially when its concentration and the surrounded temperature increased but with organic substances or low degree of temperature its activity decreased except sodium hypochlorite and sodium hydroxide which were less affected. Sodium hypochlorite and sodium hydroxide are preferred with dirty conditions or at low temperatures. Actually, the two disinfectants are often selected due to its lower price and low toxicity.

The result of Nano disinfectants against *Brucella mellitensis*:

By trying of some types of Nano disinfectants to evaluate its efficacy against *Br.melitensis* the result was as following; the effect of Dettol and Glutaraldehyde was increased when combined with silver-NPs while calcium-NPs had lower effect especially with presence of organic matters as showed in Figure (2).

Nano disinfectants had good reduction rate at low temperature even with presence of organic matters specially Glutaraldehyde with silver-NPs and Dettol with silver-NPs which had the highest reduction rate as showed in Figure (4). That agree with **Hossain *et al*., (2014)** who reported that some Nano elements can be used as disinfectants because it have antimicrobial properties and low possibility of harmful effect of the byproducts of disinfection which produced during traditional disinfection process**.**

Our results also agree with these **of Shin *et al*., (2007)** who mentioned that silver-nano particles have a good bactericidal effect and can be a good disinfectant against many types of bacteria. Various Nano-materials like carbon nanotubes, Ag, Au, CaO, ZnO, TiO2, chitosan, cationic peptides, etc. possess antimicrobial activities and therefore have been used for the treatment of infectious diseases **(Kalaiyarasan *et al*., 2017).**

Nano disinfectant has a great efficacy on bacteria as it interferes with their cellular membrane integrity, metabolic processes and morphology. The antimicrobial activity of nanostructures may be interestingly investigated in the near future owing to their high surface/volume ratio, large inner volume and other unique chemical and physical properties **(Dizaj *et al*., 2015).** Moreover, that the disinfectant solution of Glutaraldehyde 2.4 % containing silver-NPs 512 mg/liter killed 100% of *E- coli*, *Staph. aureus* and *Candida albicans* on cloth strips. More else, The germicidal efficacy of its stock solution did not changed significantly after storing at 54℃ for 14 days but, presence of organic substance made its germicidal efficacy decreased **(Shuhua *et al*., 2000).**

Calcium oxide nanoparticles and calcium hydroxide-NPs can be used as antibacterial agents as it prevent the growth of bacteria at surfaces that coated with it (**Louwakul *et al*., 2017).** Mono oxide ions as calcium oxide and magnesium oxide are very effective against large number of Gram positive bacteria and Gram negative bacteria as well as spores and it stay effective for long time and in different environmental conditions **(Stoimenov *et al*., 2002).**

However, the previous results of some researcher disagree with our opinion as nano particles can't be safely used for disinfection because it have some disadvantages as toxicity and suspected carcinogenicity to animals and human. It may also produce a new generation of more resistant bacteria to disinfectant **(Sökmen *et al*., 2001; Hajipour *et al*., 2012).**

Conclusion:

All types of used disinfectant were effective against *Brucella melitensis*. The efficacy of disinfectants influenced with increasing the contact time, concentration and temperature, but the efficacy of disinfectant decreased with presence of organic matters and at low temperature. The Nano type of disinfectants had a good efficacy against *Brucella melitensis* and its efficacy decreases to a lesser extend with presence of organic matters and low temperature so it needs more evaluation to its efficacy and if it safe for application in dairy farms.

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Figures

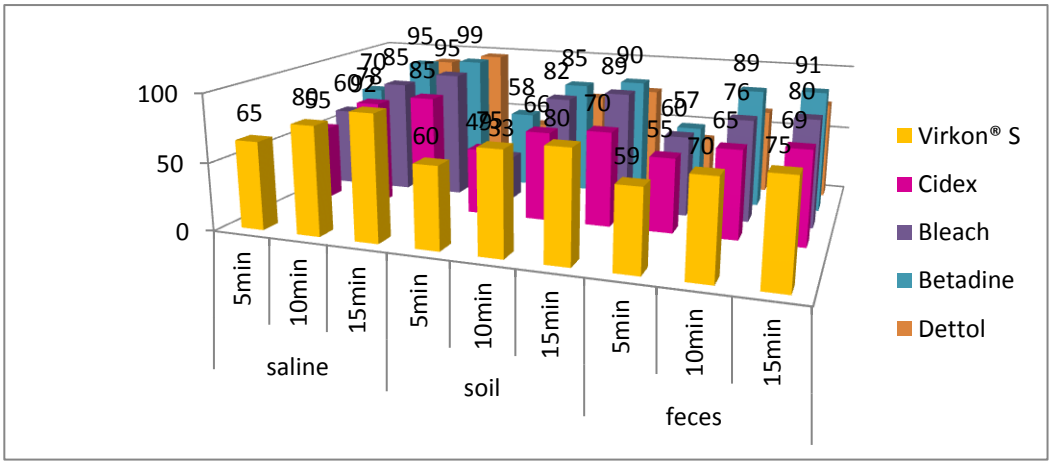

Figure (1) the reduction rate for each type of traditional disinfectants in different conditions with different times.

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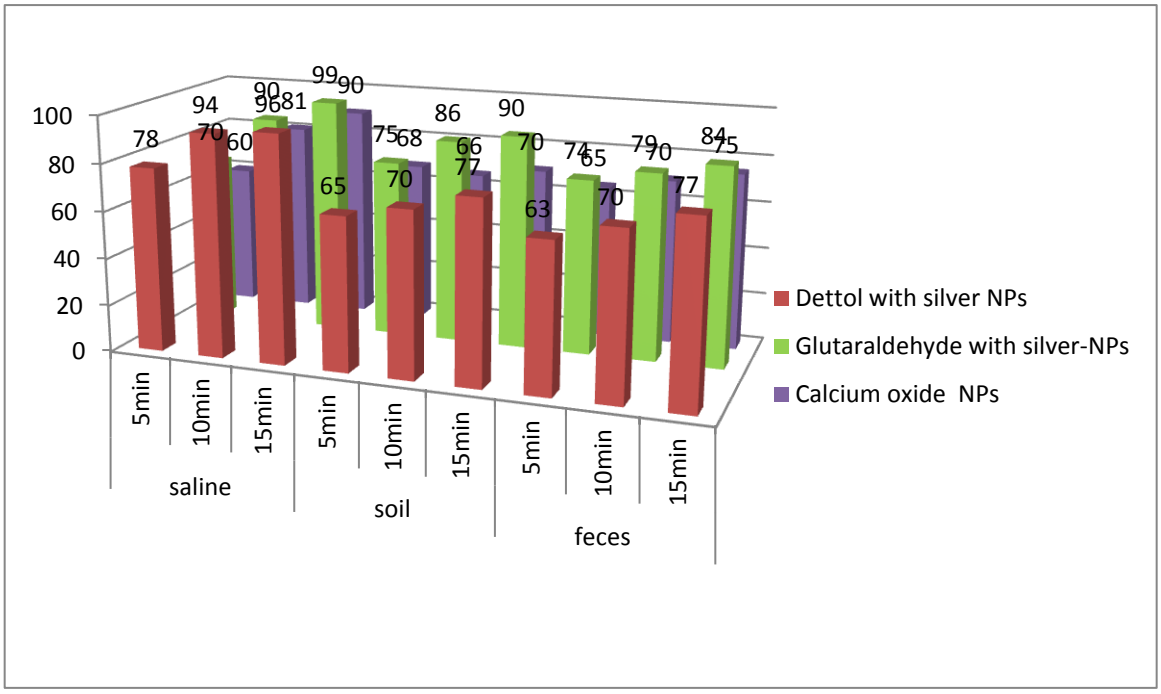

Figure (2) reduction rate of nano disinfectants in different condition and different times.

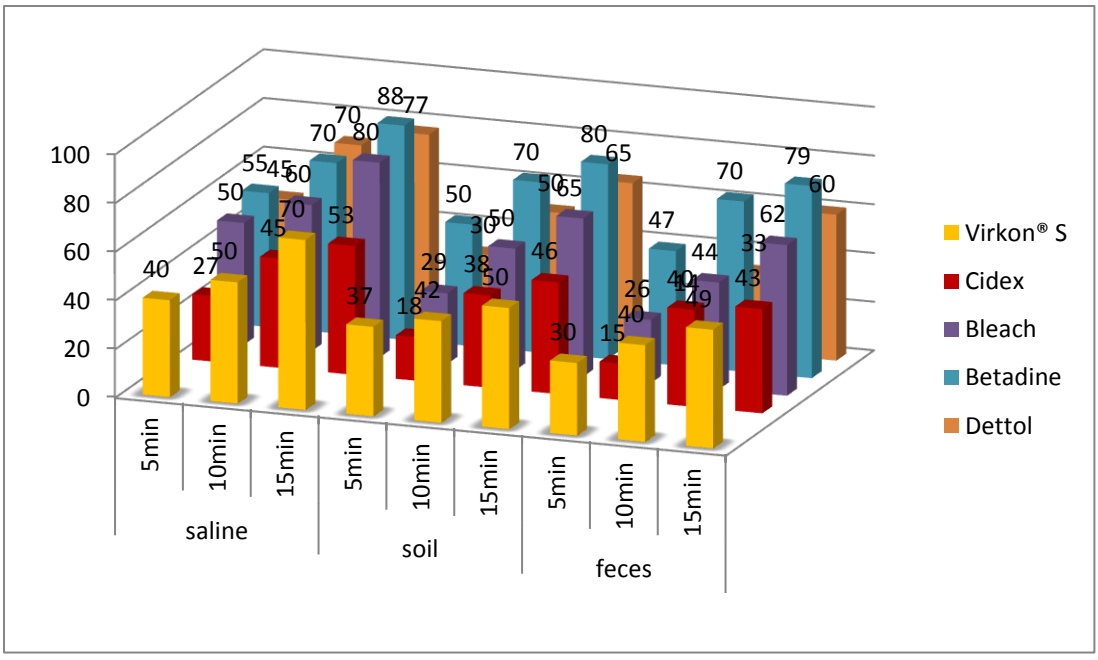

Figure (3) reduction percent of colony count at low temperature.

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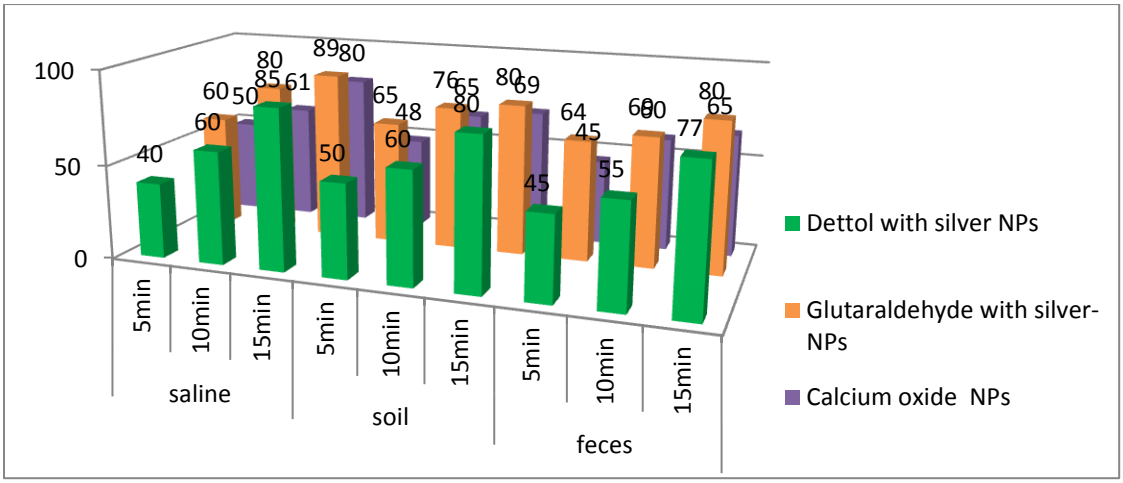

Figure (4) the reduction rate of Nano disinfectants at low temperature.

Tables:

Table (1): Disinfectants and antiseptics used in this study.

Commercial name	Active ingredient	Recommended concentration	Application
Virkon® S	Potassium peroxy monosulfate and sodium chloride	1%	Animal house and equipments
Cidex	Glutaraldehyde	2.4%	Equipments
Bleach	Sodium hypochlorite	2g/L	Biological material smooth surfaces
Betadine	Povidone iodine	1%	Skin and mucous membranes.
Dettol	Chloroxylenol (phenol)	1%	Skin of workers and skin of animals
Dettol with Silver- NPs	Chloroxylenol & Ag-NPs	1% / 100 ppm	Animal house and equipments.
Glutaraldehyde with silver-NPs	(C5H8O2) &Ag-NPs	2.4% / 100ppm	Animal house and equipments.
Calcium oxide NPs	Cao nanoparticles	100 ppm	Animal house and equipments.

Table (2) MIC&MBC of each type of disinfectants at 37°C.

disinfectants	Vircon S	Cidex	bleach	Betadine	Dettol	Dettol & Silver-NPs	Glutaraldehyde & silver-NPs	Calcium oxide NPs
MIC at 37°C	0.0750%	0. 125%	0.01%	0.63%	0.250%	0.065%	0.030%	50ppm
MBC	0.088%	0. 125%	0.1%	1%	0.9%	0.07%	0.030%	65ppm

Table(3) ANOVA analysis of reduction rate of all used disinfectant at temp 25°C

Disinfectant	Mean	Std. Dev.	Std. Error.	F-statistic value	P-value value
Virkon® S	71.6667	10.4083	6.0093	0.78791	0.49678
Cidex	61.6667	11.1505	6.4377	0.76735	0.50496
Bleach	68	30.5123	17.6163	0.24561	0.78972
Betadine	77.6667	17.2143	9.9387	0.31337	0.74226
Dettol	60.67	18.3394	10.5883	1.49699	0.29689
Dettol with Silver NPs	66	14.4222	8.3267	1.92742	0.22569
Cidex with silver-NPs	70.3333	30.6649	17.7044	0.20663	0.81888
Calcium oxide NPs	62	10.583	6.1101	0.97596	0.42957

Table(4) ANOVA analysis of reduction rate of all used disinfectant at temp 5°C

Disinfectant	Mean	Std. Dev.	Std. Error.	F-statistic value	P-value value
Virkon® S	43	6.5574	3.7859	1.24635	0.35263
Cidex	34	14.4222	8.3267	0.34156	0.72363
Bleach	48	18.0831	10.4403	1.05993	0.40347
Betadine	66.6667	15.2753	8.8192	0.10146	0.90504
Dettol	48.3333	17.5594	10.1379	1.6106	0.27548
Dettol with Silver NPs	63.34	17.5594	10.1379	0.57453	0.59116
Cidex with silver-NPs	59.3333	25.7164	14.8474	0.34505	0.72137
Calcium oxide NPs	56.6667	11.5036	6.6416	0.76326	0.50661

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