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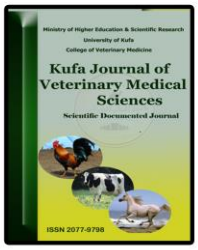

In vitro study of antibacterial and antifungal activity of some common antiseptics and disinfectants agents

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Abstract

This current study included the antifungal and antibacterial effects of antiseptics or disinfectants agents (4% formalin, 10% Dettol®, 0.5% NaClO, 70% Ethanol alcohol, 1% Iodine and 10% Potassium permanganate) against *Aspergillus flvus* (Model of Mold) ,*Candida albicans* (Model of yeast) , *E.coli* (Model of G- Bacteria) and *Bacillus thuringiensis* (Model of G+ Bacteria) , where several laboratory tests were used to evaluated the antimicrobial activity of antiseptics and disinfectants agents that used in the this study include Minimum inhibitory concentration test, Sensitive test, Zone growth diameter test and Inhibition zone test . The results showed that formalin was his effectiveness on all microbes used, while the Dettol® has been more effective than formalin in their effect on fungus and less effective on . The remainder of disinfectants and Antiseptics (Iodine, Sodium Hypochlorite, Ethanol alcohol, Potassium Permanganate) were least effective in comparison with Formalin and Dettol® on microbes used .

Keywords: ***In vitro* , antibacterial , antifungal , antiseptics and disinfectants**

دراسة مختبرية للفعالية المضادة للبكتريا و الفطريات لبعض المواد المعقمة و المطهرة الشائعة

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الخلاصة:

تضمنت الدراسة الحالية التأثير المضاد للفطريات و البكتريا لمواد مطهرة او معقمة (4% فورمالين و 10% ديتول 0.5% قاصر و 70% كحول اثيلي و 1% يود و 10% برمنغنات البوتاسيوم) ضد الرشاشية الصفراء (كنموذج للفطريات) و المبيضات البيضاء (كنموذج للخمائر و الاشريشيا القولونية (كنموذج للبكتريا السالبة لصبغة كرام) و العصوية التورنجية (كنموذج للبكتريا الموجبة لصبغة كرام) حيث استخدمت عدة فحوصات مختبرية لتقييم الفعالية المضادة للجراثيم للمطهرات و للمعقمات التي استخدمت في هذه الدراسة و التي تضمنت اختبار اقل تركيز مثبط و اختبار الاحساسية و اختبار النمو الشعاعي الدائري و اختبار القطر التثبيط. أظهرت النتائج بأن الفورمالين كان له فعالية على جميع الجراثيم المستخدمة , أما الديتول فقد كان أكثر فعالية من الفورمالين في تأثيره على الفطريات و اقل فعالية على البكتريا , أما باقي المطهرات و المعقمات (اليود و القاصر و الكحول الاثيلي و برمنغنات البوتاسيوم) كانت اقل فعالية بالمقارنة مع الفورمالين و الديتول على الجراثيم المستخدمة.

Introduction

Antiseptics and disinfectants are non-selective, anti-infective agents that are applied topically. Their activity ranges from simply reducing the number of microorganisms to within safe limits of public health interpretations, to destroying all microorganisms on the applied surface **(1).** Antiseptics are biocides that destroy or inhibit the growth of microbes in or on living tissue while disinfectants are similar but generally are biocides that are used on inanimate objects or surfaces **(2).** Antiseptics and disinfectants are used healthy sectors and care centers to control of microbes contamination on the living body tissues and inanimate objects **(3)**. Antiseptics and disinfectants agents have broad-spectrum antimicrobial activity; however, Till now there are little information about the mode of action of these agents in comparison to antibiotics.

The widespread use of these agents has control on some expectation on the development of bacterial, fungal and virus resistance **(2).**

Because of the widespread use of these substances in life, Therefore present study aimed to detect the best antibacterial and antifungal activity of some common antiseptics and disinfectants agents [Iodine, Sodium Hypochlorite, Ethanol alcohol, Potassium Permanganate, Formalin and Chloroxylenol (Dettol®)].

Materials and Methods

Antiseptics and disinfectants agents

Six agents of most common antiseptics or disinfectants agents were used in public life include 4% formalin, 10% Chloroxylenol (Dettol®), 0.5% NaClO, 70% Ethanol alcohol, 1% Iodine and 10% Potassium permanganate. All of these agents were used to evaluate the best activity as antifungal and antibacterial when used in the laboratory, animals fields , hospital or other medical uses.

Stock solution preparation

All agents used in this study were prepared from original stock solution according to V1C1=V2C2 equation **(4)**. Original stock solutions were 40% formalin, 10% Dettol® , 6% NaClO, 99.9% v/v [78.82% w/v] Ethanol alcohol [99.9% Ethanol equal to 78.82gm which resulted from each one ml of Ethanol alcohol contain 0.789mg) **(5)**, 10% Iodine and 25% Potassium permanganate. Distilled water was used as solvent for prepare these agents.

Microbe's isolates

Aspergillus flvus (Model of Mold) and *Candida albicans* (Model of yeast) isolates were taken from microbial banking which follow to Microbiology lab. /Community health Dept. /Babil institute technique, while as *E.coli* (Model of G- Bacteria) and *Bacillus thuringiensis* (Model of G+ Bacteria) isolates were taken from Central Health Laboratory of the Babel health Department.

Spore counts

Aspergillus flvus spores count was calculated according to **(6)**. After *A. flvus* was cultured on the SDA medium at 27 ºC for 5 days, Conidia were collected and suspended in five ml of sterile normal saline. The spore concentration in the suspension was determined by using a haemocytometer method which include One drop of the suspension was added into hemocytometer chamber, spores were calculated under high power 40X of light microscope using the following equation **(7)**:- $\text{Spore number/ml} = \frac{Z \times 4 \times 10^6}{N}$

Where:-Z= total number of counted spores (Spores number in 5 small square of RBCs count). N= total number of small squares (5 small square of RBCs count x 25 small square in each small square of RBCs count =80).

Final spore suspension should be obtained to equal to 10^7 spore /ml.

Yeast and bacterial counts

Spectrophotometric method was used to determine the *Candida albicans* **,** ***E.coli and Bacillus thuringiensis*** count by adding five ml of sterile distilled water to cultured media (colonies aged 24 hrs. at 37^{o}C) and mixed well with colonies then calculated the turbidity solution (Result from distilled water mixed with harvested bacteria or yeast) by serial test tube and adjusted accordance to the absorbance of 0.08-0.10 at 625nm corresponding to 5 x 10^6 CFU/ml **(8)**.

Minimum inhibitory concentration (MIC) of antiseptics and disinfectants

Tube dilution Method **(9)** was used to determine the MIC value for antiseptics and disinfectants agents that used in the current study.

Ten test tubes with eight ml of Sabouraud Dextrose Broth (SDB) for *Aspergillus flvus and Candida albicans* and 8 ml Nutrient broth for *E.coli and Bacillus thuringiensis* in each were taken and autoclaved . To the first tube, 2 ml of the each concentration (40% formalin, 10% Dettol, 6% NaClO, 100% Ethanol alcohol, 10% Iodine and 10% Potassium permanganate) was added and serial double fold dilution was done up to the 10 tube and from the 10 tube, 2 ml of the mixture was discarded. To each tube 100µl of inoculums (*C. albicans , E.coli and Bacillus thuringiensis* suspention (5 x 10^6 CFU/ml) and *A. flvus* (Spores 1x10^7 spore/ml) were added and mixed well. Test tubes were incubated for 24 hrs. at 37ºC for *C. albicans , E.coli and Bacillus thuringiensis* and 5 days at 28ºC for *A. flvus.* The least concentration of antiseptics and disinfectants agents capable of inhibiting the fungal growth was considered MIC.

Sensitive test

The effect of different antiseptics and disinfectants agents on the bacterial [*E.coli* (Model of G- Bacteria) and *Bacillus thuringiensis* (Model of G+ Bacteria)] and fungal strains [*A.flvus* (Model of Mold) and *C. albicans* (Model of yeast)] addressed by this study was assayed by agar well diffusion method **(10) .**

***Aspegillus flvus* sensitive test**

One hundred µl of *Aspegillus flvus* spore suspension were spread uniformly over SDA medium by using the spreader, then left for one hour to dry of spores on the media surface. By using cork borer, six wells (digs) were worked on the SDA media. One hundred microliter was taken from each antiseptics and disinfectants solution that has been prepared previously and put in these wells (Each well filled with one of the materials that have been studied). Same previous steps were used again for distilled water which considered as control group. Number of petri-dish for each agent repeated 5 times.

Inhibition activities of the antiseptics and disinfectants agents were determined by measuring the zones inhibition formed around the discs in millimeter. The plates were observed for presence of zones of inhibition around the discs after 7 days **(11).**

***Candida albicans , E.coli and Bacillus thuringiensis* sensitive test**

One hundred µl were taken from *Candida* ***albicans, E.coli and Bacillus thuringiensis*** standard solution (5 x 10^6 CFU/ml) and spread uniformly over SDA medium (*C.albicans*) and Nutrient agar media (***E.coli and Bacillus thuringiensis***) by using the spreader, then left for one hour to dry of cells on the media surface. By using cork borer, five wells (digs) were worked on the cultured media.

One hundred microliter was taken from each antiseptics and

disinfectants solution that has been prepared previously and put in these wells (Each well filled with one of the materials that have been studied). Same previous steps were used again for distilled water which considered as control group. Number of petri-dish for each agent repeated 5 times.

Inhibition activities of the antiseptics and disinfectants agents were determined by measuring the zones inhibition formed around the well in millimeter. The plates were observed for presence of zones of inhibition around the discs after 24hrs. at 37ºC (*C. albicans* **(12)** and *E.coli and Bacillus thuringiensis* **(13)**.

Growth inhibitory assay of antifungal effects of antiseptics and disinfectants agents

According to V1C1=V2C2 equation, original antiseptics and disinfectants agents (40% formalin, 10% Dettol®, 6% NaClO, 99.9% Ethanol alcohol, 10% Iodine and 25% Potassium permanganate). were mixed with SDA media after sterilization (Autoclave temperature 121ºC , For 15 mints at 15 Ibs) to obtain on 4% formalin, 10% Dettol® , 0.5% NaClO, 70% Ethanol alcohol, 1% Iodine and 10% Potassium permanganate then five mm diameter discs of *A.niger* mycelia were cut by sterilized cork borer from the periphery of 7 day old culture and transferred aseptically in the center of SDA media contains different antiseptics and disinfectants agents according to a pre-prepared concentrations. All petri plate including control and experimental were incubated at 28^{o}C for 7 days. After 7 days of incubation, observations were recorded and measurement of radial growth of *A. flvus* **(11).**

Note: When mixed 70% Ethanol alcohol with SDA media [It is composed from 40g/L dextrose , 10 g/L peptone and 20 g/L agar **(14)** will be mass formation(result from denaturation the peptone protein by Ethanol alcohol**(15)** and to solve this problem, we've divided the SDA media after cool in the petri dish in the form of circular rings by cork borer and then was added five ml of 70% Ethanol alcohol and left the petri-dish for 24 hrs. for diffused the 70% Ethanol alcohol between circular rings, this method modify by researcher , figure (1).

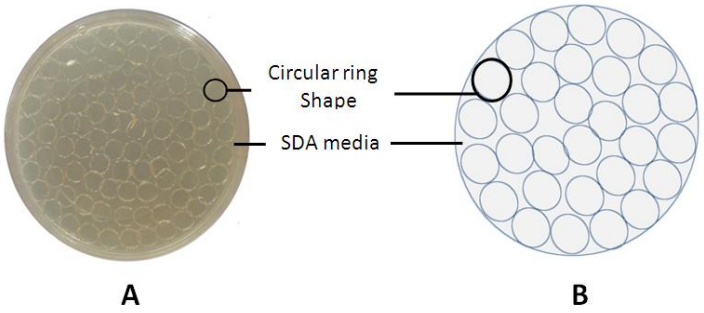


Figure (1):-SDA media contains circular rings shape in the form of hives that worked by cork borer.

A: - Circular rings shape in the SDA media.

B: - Illustration shows the circular rings in SDA media.

Statistical analysis

Statistical analysis of the experimental results were conducted according to Statistical Package for the Social Sciences (SPSS) version 13.00 where one way ANOVA was used to assess the significance of changes between experimental groups. The data were expressed as Mean ± Standard Errors (SE) and P-value<0.05 was considered statistically significance. LSD was carried out to test the significance levels among means of treatments **(16).**

Results and discussion

The results of the current study were showed that different types of microorganisms (*Aspegillus flvus , Candida albicans , E.coli and Bacillus thuringiensis*) vary in their response to different types of antiseptics and disinfectants. Where Formalin and Dettol® showed highly effective against microorganism, While Iodine, Sodium Hypochlorite, Ethanol alcohol and Potassium Permanganate showed least effective against same microorganism according to different types of laboratory tests (MIC, Sensitive test, Zone growth diameter and Inhibition zone) were used to evaluate the effects of antiseptic and disinfectants on bacteria and fungi were used in this study. The varieties effects of antiseptic and disinfectants agents against microorganism may be result from different mechanism of action of these agents on the cell structures of microorganism.

Effects antiseptic and disinfectants agents as antifungal

In this study, the antifungal effects of formalin and Dettol® were more potent than other agents (Iodine, Sodium Hypochlorite, Ethanol alcohol and Potassium Permanganate) according to laboratory tests ((MIC, sensitive test, Zone growth diameter and inhibition zone) that used to evaluate the effects these agents as antifungal, table (1 and 2) ,figure (1,2 and 3).

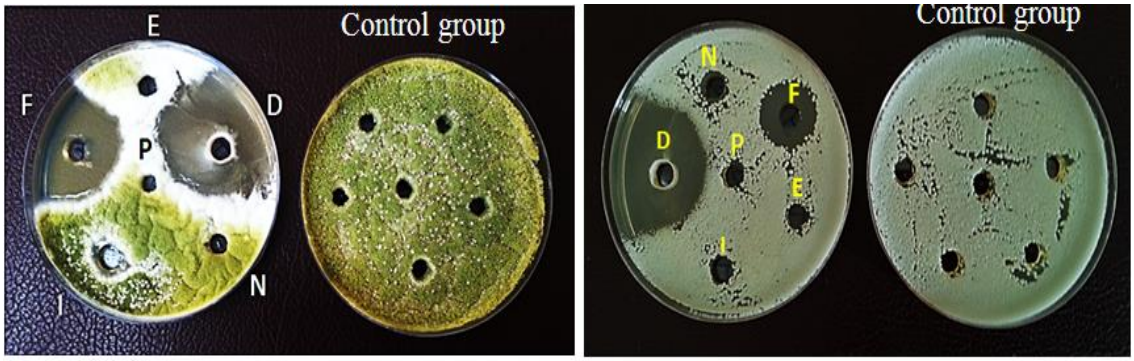
Table (1):-MIC value for Antiseptics and Disinfectants agents against ***A. flvus*** (The age of colonies 7 days at 28 ºC) and ***C. albicans*** , the age of colonies 24 h at 37 ºC) in the SDB media.

Antiseptics and Disinfectants agents	MIC mg/ml	
	A. flvus	***C. albicans***
Formalin	0.128	0.0256
Dettol®	0.00016	0.000032
NaClO	12	2.4
Ethanol alcohol	157.64	31.528
Iodine	4	20
Potassium permanganate	50	10

Table (2):-The inhibition zone diameter of different Antiseptics and Disinfectants agent against ***A. flvus*** (The age of colonies 7 days at 28 ºC)and ***C. albicans*** , the age of colonies 24 h at 37 ºC) in the SDA media.

Antiseptics and Disinfectants agent	Diameter of zone of inhibition (mm) M±SE	
	A. flvus	***C. albicans***
Formalin 4%	32.60±0.67 A	20.60±2.81 A
Dettol® 10%	36.00±0.54 A	42.80±0.24 B
NaClO 0.5%	0.00±0.00 B	0.00±0.00 C
Ethanol alcohol 70%	0.00±0.00 B	0.00±0.00 C

Iodine 1%	5.40±3.34 C	0.00±0.00 C
Potassium permanganate 10%	0.00±0.00 B	0.00±0.00 C
Control group	0.00±0.00 B	0.00±0.00 B



Aspergillus flvus LSD=3.13

Candida albicans LSD=2.57

Figure (2):-The Inhibition Zone diameter (**Dig-diffusion method**) for different antiseptics and disinfectants as antifungal agents against *A. flavus* in the SDA media for 7 days at 28 °C and *C. albicans* in the SDA media for 24h at 37 °C [F= 4% Formalin, D= 10% Dettol®, N= 0.5% NaClO, E=70% Ethanol alcohol, I= 1% Iodine and P=10% Potassium permanganate], in comparison with control group (Distilled water only).

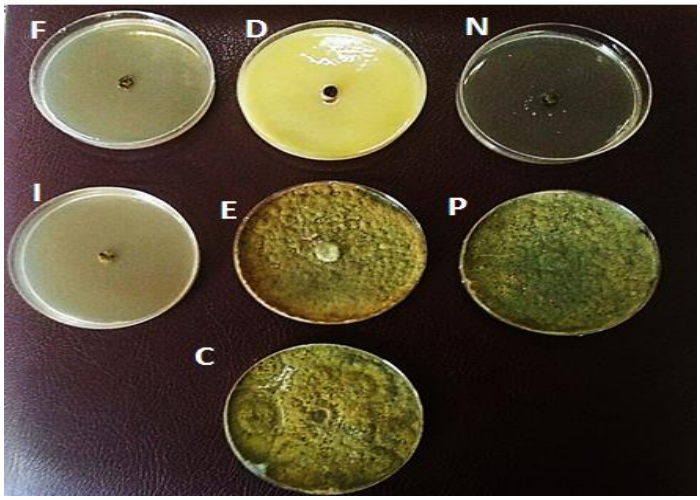
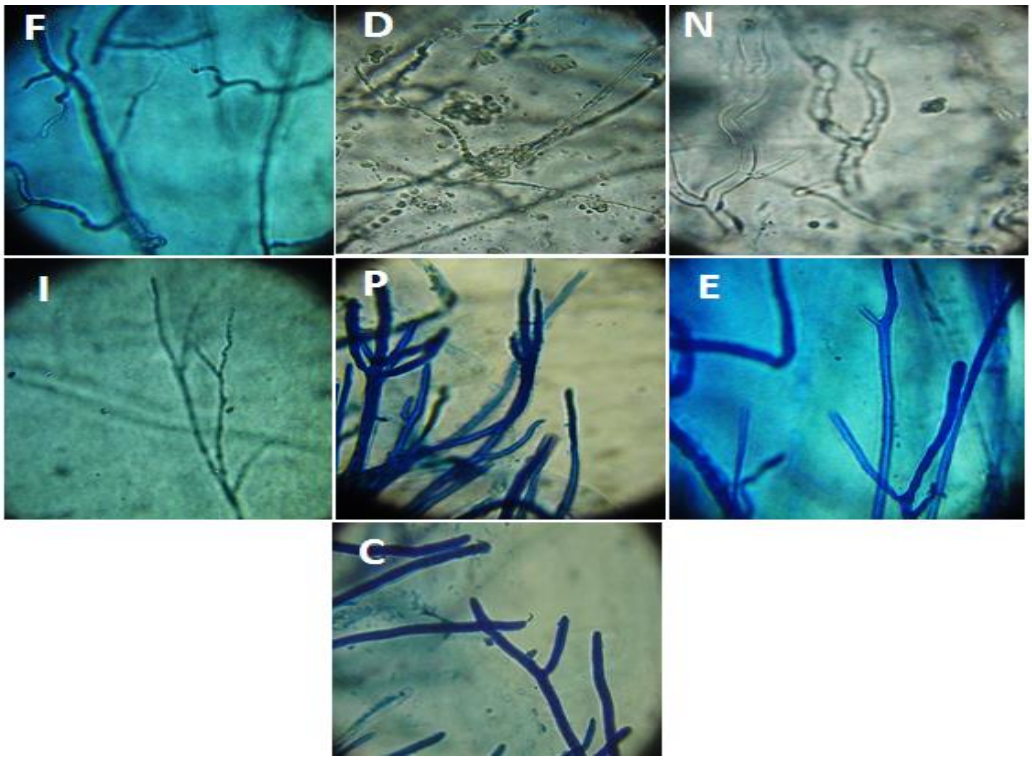


Figure (3):-The Growth Zone diameter of *A .Flvus* on SDA media which contains different antiseptics and disinfectants agents in the SDA media for 7 days at 28°C [F= SDA media contains 4% Formalin, D= SDA media contains 10% Dettol, N= SDA media contains 0.5% NaClO, E= SDA media contains 70% Ethanol alcohol, I= SDA media contains 1% Iodine and P= SDA media contains 10% potassium permanganate], in comparison with control group (Distilled water only).

The results of microscopic appearance were seen the deformity in the growing tops of mycelia for *A. flvus* cultivated on the SDA media which containing 4% Formalin (Growing tops of mycelia were tortuous with thickness of conidiophores), 10% Dettol® (Growing tops of mycelia and conidiophores were segmented), 0.5% NaClO (Growing tops of

mycelia and conidiophores were thickness and wavy) and 1% Iodine (Growing tops of mycelia and conidiophores were weakness , while *A. flvus* cultivated on the SDA media which containing 70% Ethanol alcohol and 10% Potassium permanganate were same as to *A. flvus* cultivated media which containing distilled water , figure (4).



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igure (4):-Microscopic appearance of *A .Flvus* growing tops on SDA media which contains different antiseptics and disinfectants agents in the SDA media for 7 days at 28°C [F= SDA media contains 4% Formalin, D= SDA media contains 10% Dettol®, N= SDA media contains 0.5% NaClO, E= SDA media contains 70% Ethanol alcohol , I= SDA media contains 1% Iodine and P= SDA media contains 10% potassium permanganate], in comparison with control group (Distilled water only).

Effects antiseptic and disinfectants agents as antibacterial

The antibacterial activity of formalin against G-ve (*E.coli*) and G+ve (*Bacillus thuringiensis)* bacteria were more potent than other agents (Iodine, Sodium Hypochlorite, Ethanol alcohol and Potassium Permanganate) that used in this study, while the antibacterial activity of Dettol® were effective against the G+ ve but not G-ve bacteria, according to laboratory tests (MIC and sensitive test) that used to evaluate the effects these agents as antibacterial, table (3 and 4), figure (5).

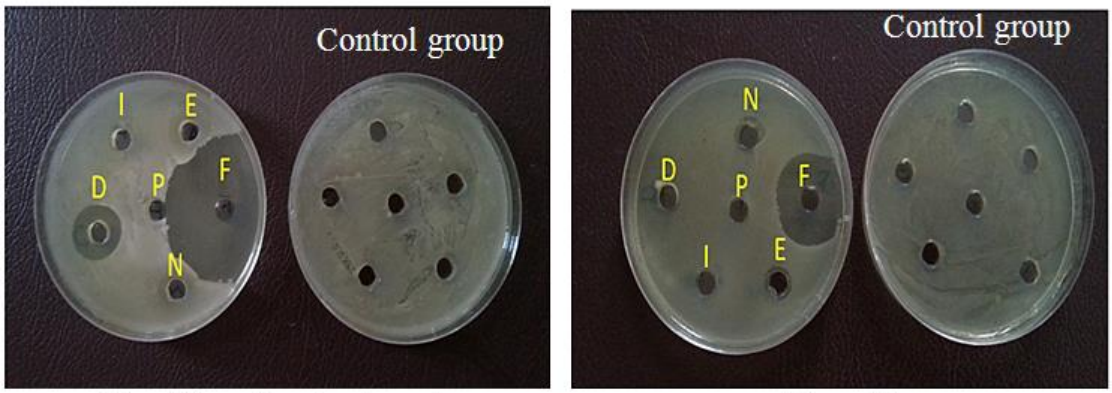
Table(3):-MIC value for Antiseptics and Disinfectants agent against ***Bacillus thuringiensis*** and ***E.coli*** in the nutrient broth media. The age of colonies 24 h at 37 ºC.

Antiseptics and Disinfectants agents	MIC mg/ml	
	Bacillus thuringiensis	***E.coli***
Formalin	0.0256	0.128
Dettol®	0.000016	0.000064

NaClO	0.096	12
Ethanol alcohol	31.528	31.528
Iodine	0.16	4
Potassium permanganate	10	10

Table (4):-Zone inhibition diameter of different Antiseptics and Disinfectants agent against ***Bacillus thuringiensis*** and ***E.coli*** in the nutrient agar media. The age of colonies 24h at 37 ºC.

Antiseptics and Disinfectants agent against	Diameter of zone of inhibition (mm) M±SE	
	Bacillus thuringiensis	***E.coli***
Formalin 4%	47.00±0.63 A	33.80±0.37 A
Dettol® 10%	23.60±3.86 B	0.00±0.00 B
NaClO 0.5%	0.00±0.00 C	7.40±1.93 C
Ethanol alcohol 70%	0.00±0.00 C	11.00±0.44 D
Iodine 1%	0.00±0.00 C	0.00±0.00 B
Potassium permanganate 10%	0.00±0.00 C	0.00±0.00 B
Control group	0.00±0.00 C	0.00±0.00 B
	LSD=3.56	LSD=1.84



Bacillus thuringiensis *E. coli*

Figure (5):-The Inhibition Zone diameter (Dig-diffusion method) of different antiseptics and disinfectants as antibacterial agents against *Bacillus thuringiensis* and *E. coli* in the nutrient agar media for 24 h at 37 °C [F= 4% Formalin, D= 10% Dettol®, N= 0.5% NaClO, E=70% Ethanol alcohol, I= 1% Iodine and P=10% potassium permanganate], in comperism with control group (Distilled water only).

The results of this study were showed the potency of Formalin and Dettol® as antimicrobial effects may be resulted from several causes, first caused, the formalin and Dettol® are broad spectrum antimicrobial activity ***(17)*** , formalin preserves or fixes tissue or cells by irreversibly cross-linking primary amine groups in proteins with other nearby nitrogen atoms in protein or DNA through a $-CH_2-$ linkage **(18) ,** While Chloroxylenol (Dettol®) also known as 4-chloro-3,5-dimethyl-hydroxybenzene, parachlorometaxylenol, 4 chloro 3,5-dimethyl phenol, 4 chloro 3,5 xylenol, and 4 chloro meta xylenol) is a substituted phenol with a molecular formula of C_8H_9ClO . Its mechanism of antimicrobial action is by the denaturation of proteins and inactivation of enzymes in the microorganisms, alters the permeability of the cell membrane that could result in the uncoupling of oxidative phosphorylation, inhibition of active transport, and loss of pool metabolites due to cytoplasmic membrane damage **(19)** . Mechanism of action of Formalin and Dettol® makes the most of this materials effective on the microbes more than other antiseptics and disinfectants.

In this study *E.coli* was resistance to Dettol®, in comparison with *Bacillus thuringiensis* was sensitive to Dettol® , figure (4), this result agreement with **(20)** and **(21)** whose reported that Gram-negative bacteria are generally more resistant to antiseptics and disinfectants than are the nonsporulating, non-mycobacterial Gram-positive bacteria. Grams negative bacteria were more resistance to disinfectant relation to Gram positive bacteria this resistance may be come from widespread use of disinfectant products, the development of resistance to antimicrobial agents, particularly cross resistance to antibiotics **(2).** As a result, resistance can be either a natural property of an organism (intrinsic) or acquired by mutation or acquisition of plasmids (Self-replicating, extrachromosomal DNA) or transposons (Chromosomal or plasmid integrating, transmissible DNA cassettes). Intrinsic resistance is demonstrated by gram-negative bacteria, bacterial spores, mycobacteria, and, under certain conditions, staphylococci) **(22).**

For an antiseptic or disinfectant molecule to reach its target site, the outer layers of a cell must be crossed. The nature and composition of these layers depend on the organism type and may act as a permeability barrier, in which there may be a reduced uptake **(23)**. Alternatively but less commonly, constitutively synthesized enzymes may bring about degradation of a compound. Intrinsic (innate) resistance is thus a natural, chromosomally controlled property of a bacterial cell that enables it to circumvent the action of an antiseptic or disinfectant. Gram-negative bacteria tend to be more resistant than gram-positive organisms, such as staphylococci **(24).**

In the current study the antimicrobial effects of 0.5% NaClO, 70% Ethanol alcohol , 1% Iodine and 10% Potassium permanganate against *Aspergillus flvus* , *Candida albicans* , *E.coli* and *Bacillus thuringiensis* , were low response in comparison with Formalin and Dettol® . These results may be explained as the following reasons.

The low responses of other agents (0.5% NaClO, 70% Ethanol alcohol , 1% Iodine and 10% Potassium permanganate) may be explained as the following Indeed, there are now multiple laboratory reports about the emergence of microbes resistance to biocides, often as a result of exposure to a lower sub-lethal concentration **(25).** Other caused the mechanisms which reduce microbial susceptibility to biocides 1-Intrinsic properties of bacteria conferring reduced susceptibility to biocides 2- Reduced susceptibility to biocides resulting from phenotypic changes 3- Reduced susceptibility to biocides associated with genotypic changes (acquired mechanisms) 4- Plasmid-mediated mechanisms **(26).**

Conclusion

1- Formalin agent was highly antibacterial but less antifungal activities against *Aspergillus flvus* , *Candida albicans* , *E.coli* and *Bacillus thuringiensis* .

2- Chloroxylenol (Dettol)® was highly antifungal but less antibacterial activities on the microbes used.

3- Other antiseptics and Disinfectants agents(Iodine, Sodium Hypochlorite, Ethanol alcohol, Potassium Permanganate) were least effective in comparison with Formalin and Dettol® on microbes used .

Recommendation

Study the molecular defects that result from antiseptics and disinfectants against different types of microbes .

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