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تأثير بعض المطهرات على ميكروبي كلوستريديم السبتكم والشوفياى

درية شرف ، محمد عبيد ، رقية عثمان ، عبدالسلام زكي

تم دراسة تأثير كل من الفورمالين والجير المشبع بالكلور والليزول ومركب اليود على حويصلات كل من ميكروبي الكلوستريديم السبتكم والشوفياى. وقد وجد ~~في~~ أن الفورمالين والجير المشبع بالكلور لهما تأثير جيد ، ثم يأتي بعد ذلك مركب اليود - كما وجد أن الليزول ليس له تأثير ، وأن نسبة ٥% من الفورمالين لها تأثير مميت على الحويصلات فتظهر التربة الملونة بميكروب السبتكم بعد ٤ ساعات وممن حويصلات الشوفياى بعد ١٨ ساعة ، ولكن الجير المشبع بالكلور المحتوى على ٥% كلور يميت الحويصلات لكلا الميكروبين بعد ٢٤ ساعة.

Veterinary Serum and Vaccine Research Institute,
Head of the Anaerobic Research Section Prof. Dr. Ikbal Farrag.

**EFFECTIVENESS OF SOME DISINFECTANT AGAINST
CLOSTRIDIUM CHAUVOEI AND CLOSTRIDIUM SEPTICUM**
(With One Table)

By
**DOREYA SHARAF; M.H. EBEID; ROUKAIA OSSMAN
and A.Z. HUSSEIN**
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SUMMARY

The sporicidal power of four disinfectants (formalin, chlorinated lime, lysol and active iodine) were tested against Cl.chauvoei and Cl.septicum spores.

The most effective was formalin then chlorinated lime and lastly active iodine; lysol had no effect. It was found that 5% formalin had an excellent bactericidal effect on both organisms. It disinfect soil contaminated with Cl.septicum spores after 4 hours exposure; while it destroyed Cl.chauvoei spores after 18 hours exposure.

Chlorinated lime containing 5% available chlorine was effecient for killing both organisms within 24 hours.

Active iodine 1/30 concentration, required two applications with one hour interval to destroy the spores of Cl.chauvoei and Cl.septicum during 72 and 96 hours respectively.

INTRODUCTION

The occurrence and presistance of spore forming clostridial organisms in soil had been largely overlooked as a problem in disease control. During recent years, it becomes evident that disinfection is one of most important method for disease control. BRANDLY and HUNGHER (1957) reported that clostridial spores were resistant to most disinfectants but spores were quite susceptible to oxidizing agents such as chlorinated lime. KERIMOV (1966) used 5% formalin two or three applications for disinfecting pasture and soil of sheep pens contaminated with Cl.novy and Cl.septicum. MERCHANT and BACKER (1967) found that 3% formalin was sufficient to destroy spores of Cl.chauvoei. KELSEY, et al. (1974) stated that alcoholic and watery hypochlorites were the best sporicidal agents. They used the concentration that provided 2000 P.P.m. chlorine to destroy clostridial spores within 10 minutes.

The resistance of clostridial spores to chemical disinfectants had not been well known. Therefore, this work was planned to find out the effect of the commonly used disinfectants on clostridial spores contaminating soil in Egypt.

MATERIAL and METHODS

I- Disinfectants:

Four types of disinfectants were used from which dilutions were prepared in sterile distilled water.

A- Formalin:

It was used as 5% concentration.

B- Chlorinated lime:

Freshly prepared calcium hypochlorite containing 33% chlorine. It was applied in its powder form as 1.5% and 5% active chlorine.

C- Active iodine: Commercial preparation

It was used as one part to 30 parts distilled water and was applied one and two applications one hour in between.

D- Lysol:

Aqueous solutions from lysol (50% saponified cresol) in concentrations of 5% and 10%.

II- Neutralizers:

A- Sodium meta-bisulphite 1% was used to neutralize the residual effect of formaldehyde according to BARTZOKAS, et al. (1968).

B- Sodium thio-sulphate 0.1% was applied to neutralize the effect of chlorine and iodine, MCKINNON (1974).

C- Azolectin solution neutralised the effect of cresol according to BLOCK (1977).

These neutralizers were autoclaved for 10 minutes for sterilization. Previously each neutralizer was added to spores of Cl.chauvoei and Cl.septicum and it showed no effect on these organisms.

III- Preparation of soil:

Soil was prepared and sterilized by autoclaving for 20 minutes according to EL-SAYED (1979).

Analysis of soil sample revealed 31% organic matter and 22% moisture. The pH value of soil was 7.5.

IV- Preparation of spore suspensions:

After COOPER, et al. (1960), spore suspensions were prepared from locally isolated strains. Clostridial spores were harvested, heated at 80°C for 10 minutes and checked for purity. Density of the suspension was adjusted by visual matching to Browns tube No. 10 (Burroughs Wellcome, Beckenham, London). The suspension used in tests contained at least 95% spores in total population as seen by Microscope.

V- Application of disinfectant:

Soil samples were artificially infected with spore suspensions of clostridium chauvoei and Cl.septicum (65 million spores per gram), and were exposed to the treatment with different

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dilutions of the disinfectants, mixed thoroughly and left at room temperature. Controls were done by adding some spore suspensions to soil samples and untreated with any disinfectant. After exposure time of 0.5, 1, 2, 4, 8, 18, 24, 48, 72, 96 and 120 hours to the disinfectant, samples were obtained. Samples of one gram each; representing treated soil as well as control were removed for subsequent microbial analysis. Each sample was suspended in 10ml distilled water containing the corresponding neutralizer and was shaken thoroughly. 0.1 ml of each sample was spreaded on plate of reinforced clostridial agar enriched with 10% sheep blood. Plates were incubated anaerobically at 37°C for 48 hours. If there was no growth, further incubation for five days was conducted. The results obtained was recorded as:

+++ : Heavy growth.

++ : good growth.

+ : few colonies.

- : no growth.

V- Animal inoculation:

Samples which showed few colonies or no growth were further studied by animal inoculation. 0.2 ml of 5.0% CaCl₂ was added to 0.2 ml sample and 0.2 ml of the mixture was injected intermuscularly in guinea-pigs; two guinea-pigs were used for each sample. The results of the survival and dead were compared with the corresponding ones obtained by culturing.

RESULTS

Table (1) illustrated the effect of the 4 used disinfectants on soil artificially infected with Cl.chauvoei or Cl.septicum spores.

The results showed that 5% formalin was effective to destroy spores of Cl.chauvoei after 18 hours application and soil became sterile. On the other hand, soil sample infected with Cl.septicum spores were sterilized after 4 hours application.

Results obtained after guinea pig inoculation from samples treated with formalin were paralleled with culture observations. This indicated that the time needed for killing Cl.chauvoei spores in soil was 18 hours and for Cl.septicum was 4 hours.

Lysol in a concentration of 5% or 10% had no effect on Cl.chauvoei and Cl.septicum spores and samples yielded resistant viable growth after 120 hours application (maximum application time of experiment). The only observation was the decrease in growth after 48 hours.

Animals inoculated with samples treated with lysol after 120 hours applications died due to the presence of the living spores.

Chlorinated lime in concentration 1.5% active chlorine failed to destroy the clostridial spores during the first three days while on the fourth day soil samples were completely sterile. However on using chlorinated lime containing 5% available chlorine, the clostridial spores of both organisms were completely destroyed after 24 hours. These results were confirmed with animal inoculation test.

The effect of active iodine in one application of the artificially infected soil with Cl.chauvoei and Cl.septicum was shown in table (1). The two organism survived the disinfectant for more than five days with high degree of growth but two applications with one hour interval, exhibited decrease in growth after 48 hours and soil samples (infected with Cl.chauvoei) were

sterile after 72 hours as well as after 96 hours for Cl.septicum. The obtained results of animal inoculation test were paralleled with culture results.

DISCUSSION

Selection of the effective economic disinfectant as well as the best concentration and right exposure time to destroy spores of Cl.chauvoei and Cl.septicum is a very important point in controlling blackleg and malignant oedema diseases.

Formalin in 5% concentration would be noticed to have the highest inhibitory action on spores of both organisms. It destroyed spores of Cl.septicum in four hours and that of Cl.chauvoei in 18 hours exposure. These finding agreed with the conclusion claimed by KERIMOV (1966) who used 5% formalin for two or three applications to disinfect pasture and soil of sheep pens contaminated with Cl.septicum and Cl.novyi. On the other hand MERCHANT and BACKER (1967) stated that 3% formalin was sufficient to destroy spores of Cl.chauvoei.

Spores of both organisms resisted chlorinated lime containing 1.5% active chlorine up to 72 hours application. While 5% active chlorine destroyed spores after 24 hours. The results were similar to that obtained by SMITH (1955), KERIMOV (1967), SYKES (1969) and KELSEY, et al. (1974). They stated that oxidizing disinfectants specially chlorine and chlorinated lime were the most effective sporicidal substances than the phenolic compounds and they recommended the use of chlorinated lime containing 5% active chlorine. One application of iodine compound resulted in survival of spores of the two organisms for 5 days. While after using the disinfectant twice; Cl.chauvoei and Cl.septicum spores were destroyed after three and four days respectively. This observation agreed with that of KELSEY, et al. (1974) who used 10% iodofor against spores of Cl.tetani and Cl.biferments and they found that iodofor was less efficient when compared with 2% hypochlorites. But SAFAROV (1964), KERIMOV (1966) and HANAFY (1983) used 10% iodine monochloride for two applications to disinfect soil of sheep pens contaminated with Cl.perfringens, Cl.novyi and Cl.septicum. This may be interpreted that iodine monochloride have two disinfecting powers (iodine, chlorine), while iodine active was only a single component.

Studying the effect of lysol on clostridial spores, it was found that both organisms resisted the disinfectant. These results were supported by SYKES (1969), KELSEY, et al. (1974) and TOPLY and WILSON (1975). They found that 5-10% of phenolic compounds failed to kill spores of Cl.tetani, Cl.bifermentas and Cl.sporogenes.

In conclusion, from the economic point, 5% formalin can be used to disinfect soil contaminated with Cl.chauvoei and Cl.septicum for its effectiveness and cheap price.

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Table (1)
Effect of different disinfectants on Clostridium chauvoei and Clostridium septicum

Disinfectant under test	Type of organism	Growth after contact with disinfectant/hours												
		Zero	1/2	1	2	4	8	18	24	48	72	96	120	
Chlorinated lime	active chlorine	Cl.chauvoei	+++	+++	+++	+++	+++	+++	++	++	+	+	--	-
	1.5%	Cl.septicum	+++	+++	+++	+++	+++	+++	++	++	+	+	-	-
	active chlorine	Cl.chauvoei	+++	+++	+++	+++	+++	++	+	-	-	-	-	-
	5%	Cl.septicum	+++	+++	+++	+++	+++	+	+	-	-	-	-	-
Formalin	5%	Cl.chauvoei	+++	++	++	++	+	+	-	-	-	-	-	-
		Cl.septicum	+++	++	+	+	-	-	-	-	-	-	-	-
Lysol	5%	Cl.chauvoei	+++	+++	+++	+++	+++	+++	+++	+++	+	+	+	+
		Cl.septicum	+++	+++	+++	+++	+++	+++	+++	+++	+	+	+	+
	10%	Cl.chauvoei	+++	+++	+++	+++	+++	+++	+++	+++	+	+	+	+
		Cl.septicum	+++	+++	+++	+++	+++	+++	+++	+++	++	++	+	+
Iodine active 1/30	one applica- tion	Cl.chauvoei	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
		Cl.septicum	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	two applica- tions	Cl.chauvoei	+++	+++	+++	+++	+++	+++	+++	+++	+	-	-	-
		Cl.septicum	+++	+++	+++	+++	+++	+++	+++	+++	++	+	-	-
		Cl.chauvoei	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Control		Cl.septicum	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	

+++ = Heavy growth

++ = Good growth

+ = Few colonies

- = No growth

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