RESULTS ON FUNGICIDAL ACTIVITY OF VIRKON'S DECONTAMINANT ON *Aspergillus genus*

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Abstract

The objective of this paper was to determine the fungicidal activity of Virkon'S decontaminant on a strain of Aspergillus brasiliensis ATCC 16404.

To achieve this goal, an artificial contamination of 100 cm2 surfaces was achieved; 1 strain of Aspergillus brasiliensis was used to prepare a fungal suspension with a 1 McFarland density using a Biosan density.

From the fungal suspension with the density of 1 McFarland, several work solutions were made with different concentrations of 0.5%, 1%, and 1.5%, respectively.

Several surfaces were contaminated with the suspension of Aspergillus brasiliensis, then these surfaces were sprayed with the Virkon'S decontaminant at the three prepared concentrations; contact time was 10 minutes, 20 minutes and 30 minutes, respectively.

Finally, sanitation tests were taken to verify the effectiveness of decontamination with this product.

The conclusion of our research was that Virkon'S decontaminant had ineffective fungicidal activity for the 0.5% concentration at all three contact times.

Concentrations of 1% and 1.5%, however, were effective at all contact times.

Key word: decontaminants, fungal suspension, *Aspergillus genus*

INTRODUCTION

Contamination of the environment plays an important role in the transmission of pathogens, which can endanger both human and animal health.

Effective disinfection of the environment is essential; ultrasonic systems and decontaminated substances help to reduce environmental contamination after cleaning and disinfection.[8]

In the atmospheric air there is always a varied microbial flora, the number of which varies according to the place and time of harvest, having higher values near the soil than at altitude. [7]

The biosecurity measures to be applied to broiler chickens are:

- prevention measures that address the potential risk factors on the farm (biotic and abiotic);

- organizational measures to prevent infectious and parasitic agents from entering the farm. [10]

All these measures are taken to achieve a good slaughter yield at the end of the growing season (the commercial yield value for broiler chickens must be between 80% and 84%, with an average of 82%). [9]

Virkon'S powder was the first widely known virucidal veterinary disinfectant recognized by the industry and governments around the world as the first choice disinfectant for the prevention and control of animal diseases.

Numerous studies have been carried out at international level on the action of Virkon'S decontaminant.

Broadley et al. tested the Virkon'S solution at concentrations between 2-4% in 1993 against Mycobacterium tuberculosis and Mycobacterium aviumintracellulare, the exposure time ranging from 30 to 120 minutes.

Two test procedures were used: a standard method and method involving Bactec 960 system (these systems have

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sensitivity and specificity in mycobacterial detection).

The researchers' conclusion was that Virkon'S solutions did not produce a satisfactory reduction in the experimental strains at a 60 or 120 minute exposure period.

In 1997 Ares-Mazas and others. research has been carried out on the decontaminating effects of Virkon; they were exposed to oocysts of Cryptosporidium parvum obtained from infected calves naturally in solutions with concentrations between 1-10% Virkon'S for periods of 10-360 minutes, and then inoculated intragastrically mice. [1]

Although they have not been able to eliminate the infection completely, its intensity was reduced considerably after prolonged periods of exposure (up to> 90% depending on the concentration of disinfectant used), which indicates that this product may have a value disinfectant good when when the exposure period is extended as long as possible.

In 2000, Hernandez and others have conducted a study to test in vitro the Virkon's bactericidal, fungicidal and sporicidal activity against 10 different types of microorganisms [5]

They used a 1% Virkon'S solution and demonstrated bactericidal activity against *Pseudomonas aeruginosa*, *Escherich*ia coli, *Staphylococcus aureus*, *Enterococcus hirae* and *Mycobacteriu and* antiviral activity against poliovirus. Following the results, they concluded that a 1% Virkon'S concentration is only effective against vegetative forms.

Lisa McCormick and Gargi Maheshwari (2004) verified the action of Virkon'S 0.9% solution on adenovirus types 5 and 6; the aim of the research was to determine whether this decontaminant can be successfully used in the pharmaceutical industry. [6]

The results obtained showed a considerable reduction in the presence of the two types of adenovirus after a minimum exposure of 5 minutes.

Magdalena Dunowska and others (2005) have conducted studies to determine the effectiveness of aerosolization decontamination with Virkon'S on Staphylococcus aureus and *Salmonella enterica* bacteria on various surfaces.

A quantity of broth with strains of Salmonella enterica and *Staphylococcus aureus* was enriched and then introduced into the pre-marked areas of each location and allowed to dry.

Later, aerosolization with 1% Virkon'S was performed in rooms. [4]

Sanitation samples were taken before and after aerosolization to evaluate the disinfectant activity of Virkon'S solution; a reduction in *Salmonella enterica* and *Staphylococcus aureus* bacteria was observed, especially in samples taken from non-porous horizontal surfaces that were not blocked by the airflow.

MATERIAL AND METHOD

Determination of total number of fungi on surfaces was performed according to the working standard SR ISO 21527-2 / 2009.

In the first phase artificial contamination of 100 cm2 surfaces was made using an aluminum template with the sides of 10 x 10 cm; a fungal strain of *Aspergillus brasiliensis* ATCC 16404 was used to prepare a fungal suspension.

For the preparation of the working solutions we weighed in turn 0.5 g of Virkon's powder, then 1 g of powder and 1.5 g of Virkon's powder; above these amounts was added 100 ml of distilled water (Figure 1).

Fig. 1 Preparation of Virkon'S solutions
a) weighing the powder; b) preparing the solution

In the first phase, it was checked whether the area on which the research is to be carried out is free from germs; this involved the collection of sanitation tests and the analysis of the results.

The substance with which the surface investigated was initially decontaminated was Incidin, a ready-to-use solution containing 25 g of 1-propanol / 100 ml of liquid and 35 g of 2-propanol / 100 ml. The results obtained were negative, which demonstrates that the decontamination of the respective surface was correctly performed. Subsequently, they were contaminated with a sterile sanitation pad of 100 cm^2 areas with the fungal suspension of Aspergillus brasiliensis prepared in the laboratory and the contaminated surfaces allowed to dry.

After drying, the surfaces were sprayed with the decontaminants prepared at the specified concentrations.

Another very important factor that has been taken into account was the contact time; thus, after spraying the decontaminant on the surface previously contaminated with the suspension of Aspergillus brasiliensis, it was left to act for 10, 20 and 30 minutes.

Sanitary tests were carried out before Aspergillus brasiliensis surface contamination after contamination of the surface with the fungal suspension of Aspergillus brasiliensis and after decontamination with the 3 concentrations of the decontaminant used (Virkon'S).

RESULTS AND DISCUSSIONS

Following the experiments we can draw the following conclusions:

- Virkon'S decontamination solution had ineffective fungicidal activity at the 0.5% concentration at all three contact times; on wild-type fungi (DRBC) plates, a large number of fungal colonies have developed.

Concentrations of 1% and 1.5% were effective at all contact times (Table 1 and Figures 2, 3, 4).

Table 1 Results obtained after decontamination of surfaces with Virkon'S

Substance used	Concentration (%) and contact time (minutes)	Before surface contamination with A. brasiliensis	After surface contamination with A. brasiliensis	After decontamination
Virkon'S	0,5% - 10 ′	Absent/100 cm^2	Present/100 cm^2	Present/100 cm^2
	0,5% - 20 ′	Absent/100 cm^2	Present/100 cm^2	Present/100 cm^2
	0,5% - 30 ′	Absent/100 cm^2	Present/100 cm^2	Present/100 cm^2
Virkon'S	1% - 10′	Absent/100 cm^2	Present/100 cm^2	Absent/100 cm^2
	1% - 20′	Absent/100 cm^2	Present/100 cm^2	Absent/100 cm^2
	1% - 30′	Absent/100 cm^2	Present/100 cm^2	Absent/100 cm^2
Virkon'S	1,5% - 10′	Absent/100 cm^2	Present/100 cm^2	Absent/100 cm^2
	1,5% - 20′	Absent/100 cm^2	Present/100 cm^2	Absent/100 cm^2
	1,5% - 30′	Absent/100 cm^2	Present/100 cm^2	Absent/100 cm^2

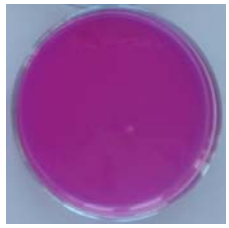

Fig. 2 DRBC medium seeded after Aspergillus brasiliensis contamination

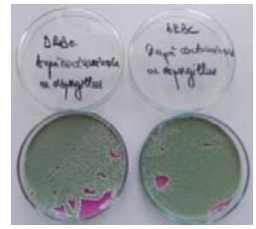

Fig. 3 DRBC media seeded after Aspergillus brasiliensis contamination

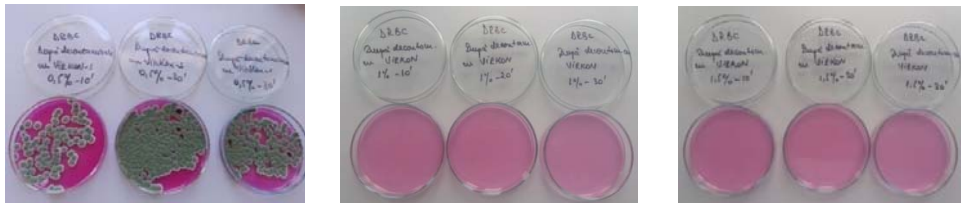

Fig. 3 DRBC media plated after decontamination with Virkon'S

CONCLUSIONS

Analyzing the results obtained, it was found that the fungicidal activity of the Virkon'S decontaminant was ineffective for the 0.5% concentration at all three contact times.

Concentrations of 1% and 1.5%, however, were effective at all contact times.

Based on the results obtained, we recommend that the surface decontamination process be carried out with a solution of Virkon'S having a concentration of at least 1%, in order to obtain appropriate results.

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