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BRIEF COMMUNICATION

ACTIVITY OF TRI-N-BUTYL TIN MALEATE IN CARPETS AGAINST *Staphylococcus aureus* AND *Aspergillus niger*, VERIFIED THROUGH TWO METHODOLOGIES: INHIBITION HALO (HZ) AND INHIBITION SURFACE (PRINT)

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SUMMARY

The aim of the present study was to verify the activity of the Tri-N-Butyl Tin maleate compound against *Staphylococcus aureus* and *Aspergillus niger*, after its industrial application in 40 samples of carpets of different materials (polypropylene, polyester, polyamide and wool). The qualitative assays were performed through two methodologies: Inhibition Halo (HZ) and Inhibition of Surface (Print). The carpet with the product inhibited 100% of bacterial (*Staphylococcus aureus*) and fungi (*Aspergillus niger*) growth, under the conditions of this study. The microbial inhibition was higher in upper portion of carpets. The methodologies employed appear to be adequate to test the bactericide and fungicide activities of the Tri-N-Butyl Tin maleate. The print methodology confirmed the results obtained by the inhibition zone assay. Further studies using the same methodologies are needed to confirm our results.

KEYWORDS: Antifungal; Antibacterial; Carpets.

INTRODUCTION

The importance of fungi causing superficial and systemic infections, and especially allergies, has grown significantly over the last decades. Anemophilous species of the genus *Aspergillus*, *Penicillium and Cladosporium* among others, play important roles in various types of allergies. They are considered the more ubiquitous aeroallergens, representing important role in the etiology of upper respiratory tract allergy, being responsible to hypersensitivity reactions in hyperactive patients[10]. *Aspergillus* spp. may cause a variety of pulmonary diseases, depending on immune status and the presence of underlying lung disease. These manifestations range from invasive pulmonary aspergillosis in severely immunocompromised patients, to chronic necrotizing aspergillosis in patients with chronic lung disease and/or mildly compromised immune systems[11]. *A. fumigatus* is the most common species of *Aspergillus* recovered from aspergillosis, being that *A. niger*, *A. flavus*, *A. nidulans*, *A. oryzae*, and *A. terreus* have occasionally been responsible for this condition[10]. Aspergilloma is mainly seen in patients with cavitary lung disease, while allergic bronchopulmonary aspergillosis is described in patients with hypersensitivity to *Aspergillus* antigens[11]. Acting as hosts of these species are, most significantly, children, the aged, and patients with propensity for these syndromes, those are debilitated or immunosuppressed[9,11].

The gram-positive cocci are the most frequently microorganisms isolated from clinical specimens in the microbiology laboratory. The distribution of these bacteria is ubiquitous and although this organism is frequently a part of the normal human microflora of the skin and other body sites, it can cause significant opportunistic infections under the appropriate conditions. *Staphylococcus aureus* is one of the most important human pathogens, possessing several virulence factors that contribute to its ability to cause disease. *S. aureus* may cause a variety of infectious processes, such as acute or chronic disease, ranging from skin infections (folliculitis, impetigo, furuncles and carbuncles) to life-threatening systemic illnesses[2,5].

Research institutions, academic centers, as well as the chemical and pharmaceutical industries, are increasingly seeking for agents to effectively aid in the area of anemophilous microorganisms. Consequently, if we have an allergenic microbiota controlled or even reduced, this fact will result in a better quality of human life, especially for patients who suffer from allergies, due to direct content or inhalation of allergens. On the other hand, an accurate methodology which does not require time-consuming and difficult procedures to investigate antibacterial and antifungal activity of substances may facilitate this research area.

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The Tri-N-Butyl Tin maleate compound is a aqueous solvent dispersible concentrate that can be added into fabrics, incorporated into adhesives, or added to latex emulsions to give protection against fungal and bacterial degradation. Microbe-inhibiting fabrics may be constructed by weaving, knitting, or otherwise forming the fabric from fibers which possess the desired microbe-inhibiting properties. There is evidence that the site of action of organotins may be both at the cytoplasmic membrane and intracellular level, in spite of their lipophilicity regarding as membrane active[9].

The aim of the present study was standardize a methodology to verify the activity of the Tri-N-Butyl Tin maleate compound against *Staphylococcus aureus* and *Aspergillus niger*, after its industrial application in samples of carpets of different materials.

METHODS

The qualitative methods were performed in two stages: Inhibition Zone (IZ - PRICE *et al.*, 1982[7]) and Inhibition of surface (Print - MARIAT & ADAN-CAMPOS, 1967[6]), for in such away were randomly collected 40 samples of fabric carpets (base of carpets and upper portion of carpet were tested), made of different materials. These samples were treated with the antifungal and antibacterial Tri-N-Butyl Tin maleate by sprinkle: PP (polypropylene), PES (polyester), PA (polyamide) and wool. All tests with the samples were carried out in three repetitions. For each repetition, two controls were run under conditions identical to those described above, with the exception that in one only untreated samples were used, while the other involved no carpet sample (Fig. 1A).

The microorganisms were selected based on the end use of the carpet and were the following: *Aspergillus niger* (ATCC/16604) - frequent airborne fungi that may cause a variety of allergy diseases, and *Staphylococcus aureus* (ATCC/25923) - common Gram-positive bacteria that is present on the skin and other body sites that may cause infectious diseases.

Inhibition Zone (IZ): Following Kirby and Bauer methodology (BAUER *et al.*, 1966[1]), *S. aureus* and *A. niger* were seeded into Petri plates containing basic medium (bacteria;Trypticase-Difco agar/fungi: agar Sabouraud dextrose-Difco), equivalent to 1-2 x 10^8 cells/mL (0.5 McFarland). After absorption of the bacteria and fungi inoculum in the solid medium, a sample of test specimen (5 X 2.5 cm) was placed in the center of the Petri plate and subsequently incubated at 37 °C, in a BOD chamber, for 24 hours, for bacteria, and 25 °C for approximately five days for fungi. Two visually results were verified and the quantitative performance obtained through calculus: IZ = SD/SDH (IZ = inhibition zone; SD = sample diameter; SDH = sample diameter more (+) inhibition halo measure. When IZ = 1, inhibition zone absence; IZ < 1, inhibition zone formed caused by some antibacterial/antifungal on the surface of the carpet sample that migrated into water based.

Inhibition of Surface (Print): *S. aureus/A. niger* was seeded into Petri plates containing basic medium (bacteria, Trypticase-Difco agar/ fungi: agar Sabouraud dextrose-Difco), equivalent to 1-2 x 10^8 cells/ mL (0.5 McFarland). After absorption of the bacteria and fungi inoculum in the solid medium a sample of carpet (4 cm X 4 cm) was placed in the center of the Petri plate and subsequently incubated at 37 °C, in a BOD chamber, for 24 hours, for bacteria, and 25 °C for approximately five days for fungi.

After this time of incubation, a sample was removed of Petri plate and printed into Petri plates containing sterile basic medium (bacteria;Trypticase agar-Difco/fungi: agar Sabouraud dextrose-Difco). A plate with Trypticase agar medium was incubated at 37 °C, in a BOD chamber, for 24 hours, and the plate with agar Sabouraud dextrose-Difco was incubated at 25 °C for approximately five days. The reading was performed in counting chamber of CFU (colony forming units), onto surface printed, and the possible obtained results were: CFU > 100, 0% inhibition (*S. aureus/A. niger* recovered all surface printed); CFU = 0, 100% surface inhibition; 100 < CFU > 1, surface inhibition calculated in percent, numbers of CFU by surface printed.

RESULTS AND DISCUSSION

The treatment with antifungal and antibacterial compounds on carpets, fabrics of mattress coverings, pillows, blankets, curtains, etc., allied with a good routine of cleanness, can help in the control of the damaging allergic reactions in closed environments, besides controlling bacteria that could cause opportunistic infections under the appropriate conditions. The product for this application must be not toxic, be correctly applied inside the allowed concentrations, to inhibit around 100% of the microorganisms to prevent an election of more resistant microorganisms, and must be duly valued.

The acaricide activity of this component industrially applied to samples of carpets, mattress foam, and fabrics was verified by UEHARA *et al*., 2006[8]. Organotins are used for industrial and agricultural purposes and as microbe-inhibiting agents, being significantly more toxic than inorganic tins. A number of bacterial processes on membrane functions can be inhibited by organotins, including effects on transduction, solute transport and retention and oxidation of substrates. The toxic effects on algae and fungi are little known[3].

Methodology for testing compounds for determination of antifungal and antibacterial activity had been developed with adaptations. Based on two simple and easy methodologies, it is possible to display precise qualitative results. The Inhibition Zone (IZ) methodology was adapted from the plate method for detection of phospholipase activity in *Candida albicans*[7]. The Inhibition of Surface (Print) methodology was based on the carpet technic, a simple method for taking samples from superficial mycosis[6]. These adapted methodologies obtained excellent application in this work.

The Tri-N-Butyl Tin maleate compound presented better activity against fungi than against bacteria (Fig. 1B). The samples 2 (polyamide) and 4 (wool) presented IZ = 0.40 (base of carpet) and IZ = 0.20, with great inhibition zone (upper portion of carpet); following samples 1 (polypropylene) and 3 (polyester) with IZ = 0.60 (base of carpet), and respectively IZ = 0.30 and 0.25 (upper portion) (Table 1). There is no significant difference of the activity against bacteria between the materials types of the samples, IZ = 0.70 (base of carpet) and IZ = 0.60 (upper portion of carpet) (Fig. 1C).

The sample 1 showed 0 mm of zone of inhibition for bacteria, IZ =

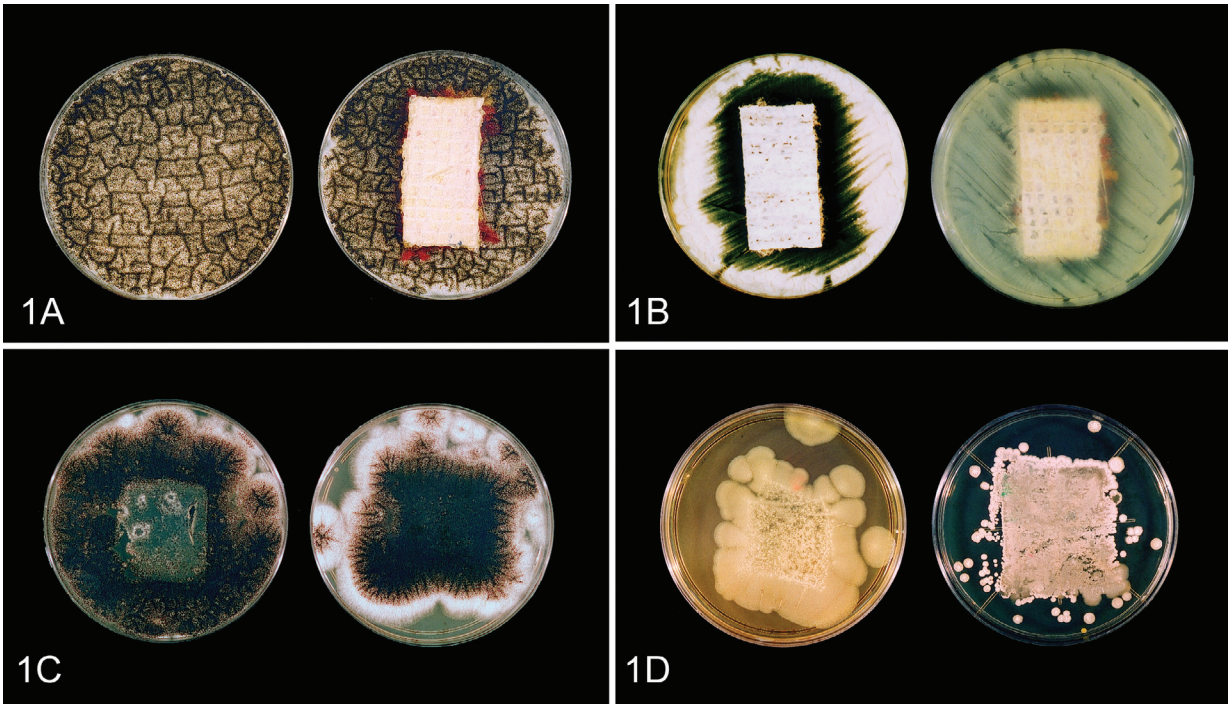

Fig. 1 - A: Control sample of *Aspergillus niger* growth and viable fungi growth on untreated control sample. **B:** Inhibition zone (IZ) for fungi, upper portion of carpet, and inhibition zone (IZ) for bacteria (*Staphylococcus aureus*), base portion of carpet. **C:** Treated sample without fungi growth on the printed surface and untreated sample with total fungi growth on the printed surface. **D:** Treated sample without bacterial growth on the printed surface and untreated sample with total bacterial growth on the printed surface. *A. niger* was incubated at 25 °C for five days, and *S. aureus* at 37 °C for 24 hours.

Table 1
Inhibition Zone - IZ values for the samples of the base and of the superior portion of the carpet incubated with bacteria and fungi

	Fungi			
Item	No. of samples	Samples	Base of carpet IZ (arithmetic mean)	Up. Port. of carpet IZ (arithmetic mean)
1	10	PP	0.60	0.30
1C	10	PP C	1	1
2	10	PA	0.40	0.20
2C	10	PA C	1	1
3	10	PES	0.60	0.25
3C	10	PES C	1	1
4	10	Wool	0.40	0.20
4C	10	Wool C	1	1
	Bacteria			
Item	No. of samples	Samples	Base of carpet IZ	Up. Port. of carpet IZ
1	10	PP	*1	0.60
1C	10	PP C	1	1
2	10	PA	0.70	0.60
2C	10	PA C	1	1
3	10	PES	0.70	0.60
3C	10	PES C	1	1
4	10	Wool	0.70	0.60
4C	10	Wool C	1	1

PP = polypropylene; PES = polyester; PA = polyamide; C = control; Up. Port. = upper portion of carpet; IZ = 1, absence inhibition zone; IZ <1 with inhibition activity.

1, and 100% inhibition of surface, in contrast with the CFU = 0 on the printed surface (Fig. 1D). All samples had 100% of inhibition of surface, with CFU = 0 (*S. aureus/A. niger)* (Table 2). Control samples showed IZ = 1 with 0% of inhibition growth *S. aureus/A. niger* in the totality of the surface. In all samples tested, the inhibition was higher in upper portion of carpets because the Tri-N-Butyl Tin maleate was sprinkled over carpets, presenting more exposition and product absorption in this portion of carpet, in comparison with the base of carpet.

Table 2
Surface inhibition-Print values for the samples incubated with bacteria and fungi

Item	No. of samples	Samples	Base of carpet CFU	Up. Port. of carpet CFU
1	10	PP	0	0
1C	10	PP C	>100	>100
2	10	PA	0	0
2C	10	PA C	>100	>100
3	10	PES	0	0
3C	10	PES C	>100	>100
4	10	Wool	0	0
4C	10	Wool C	>100	>100

PP = polypropylene; PES = polyester; PA = polyamide; C = control, CFU = 0, 100% inhibition.

The carpet treated with the product inhibited 100% of bacterial and fungi growth, under the conditions of this study. This fact was observed with 100% reproducibility in the experiments conducted, assuring that the methodology employed in the present study was adequate to test the effectiveness of the activity against *Staphylococcus aureus* and *Aspergillus niger***.** The use of the print methodology confirms the obtained results of inhibition zone assay.

The adequacy of these two traditionally praised methodologies in the mycology could be used to verify the effectiveness of different products treated with antifungal and antibacterial compounds, using other fungi and bacteria species in accordance with the final use of the tested product.

RESUMO

Atividade de tri-n-butyl tin maleate em carpetes contra *Staphylococcus aureus* e *Aspergillus niger*, verificada através de duas metodologias: Zona de Inibição (ZI) e Superfície de Inibição (Impressão)

O objetivo do presente estudo foi verificar a atividade do composto maleato de estanho tri-n-butílico contra *Staphylococcus aureus* e *Aspergillus niger*, após sua aplicação industrial em 40 amostras de carpetes de diferentes materiais (polipropileno, poliéster, poliamida e lã). Os ensaios qualitativos foram realizados através de duas metodologias: Zona de Inibição (ZI) e Superfície de Inibição (Impressão). Os carpetes tratados com o produto apresentaram 100% de inibição de crescimento bacteriano (*Staphylococcus aureus*) e fúngico (*Aspergillus niger*), sob as condições desse estudo. A inibição de crescimento microbiano foi mais elevada na porção superior dos carpetes. As metodologias empregadas parecem ser adequadas para testar a atividade bactericida e fungicida do maleato de estanho tri-n-butílico. A metodologia de impressão confirmou os resultados obtidos no ensaio de zona de inibição. Estudos futuros utilizando as mesmas metodologias são necessários para confirmação destes dados.

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REFERENCES

1. BAUER, A.W.; KIRBY, W.M.; SHERRIS, J.C. & TURCK, M. - Antibiotic susceptibility testing by a standardized single disk method. **Amer. J. clin. Path., 45:** 493-496, 1966.

2. CASEY, A.L.; LAMBERT, P.A. & ELLIOTT, T.S. - *Staphylococci*. **Int. J. antimicrob. Agents,** (suppl. 3): S23-S32, 2007.

3. COONEY, J.J. & WUERTZ, S. - Toxic effects of tin compounds on microorganisms. **J. Indian Microbiol. Biotechnol., 4**: 375-402, 1989.

4. GOMPERTZ, O.F.; GAMBALE, W.; PAULA, C.R. & CORRÊA, B. - Alergia a fungos. In: TRABULSI, L.R. & ALTERTHUM, F. - **Microbiologia**. 4. ed. São Paulo, Atheneu, 2005. p. 501-503.

5. KONEMAN, E.W.; ALLEN, S.D.; JANDA, W.M.; SCHRECKENBERGER, P.C. & WINN Jr., W.C. - **Diagnostic microbiology**. **Color atlas and textbook**. 5. ed. Philadelphia, Lippincott, 1997.

6. MARIAT, F. & ADAN-CAMPOS, C. - La techinique du carré de tapis, méthod simple de prélèvement dans les mycoses superficielles. **Ann. Inst. Pasteur**, **113:** 666-668, 1967.

7. PRICE, M.F.; WILKINSON, I.D. & GENTRY, L.O. - Plate methods for detection of phospholipase activity in *Candida albicans.* **Sabouraudia**, **20:** 7-14, 1982.

8. UEHARA, S.; FRANZOLIN, M.R.; CHIESA, S. *et al*. - Effectiveness of house dust mite acaricide tri-n-butyl tin maleate on carpets, fabrics and mattress foam: a standardization of methodology. **Rev. Inst. Med. trop. S. Paulo**, **48:** 171-174, 2006.

9. WHITE, J.S.; TOBIN, J.M. & COONEY, J.J. - Organotin compounds and their interactions with microorganisms. **Canad. J. Microbiol., 45:** 541-554, 1999.

10. ZANDER, D.C. - Allergic bronchopulmonary aspergillosis: an overview. **Arch. Path. Lab. Med., 129**: 924-928, 2005.

11. ZMEILI, O.S. & SOUBANI, A.O. - Pulmonary aspergillosis: a clinical update. **QJM, 100:** 317-334, 2007.

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