

ANTIFUNGAL PROPERTIES OF CLOVE OIL (*EUGENIA CARYOPHYLLATA*) IN SUGAR SOLUTION

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ABSTRACT

The effect of temperature, concentration and contact time on the fungicidal effect of clove oleoresin dispersed in a concentrated sugar solution at 21 and 37°C, and clove oleoresin at 0.2 to 0.8% (v/v) was studied. The test microorganisms were *Candida albicans*, *Penicillium citrinum*, *Aspergillus niger* and *Trichophyton mentagrophytes*. The fungicidal effect was enhanced at 37°C; at this temperature short contact times (e.g. 1 min.) were enough to eliminate a microbial inoculum of 10⁶ c.f.u./ml of *C. albicans*. Although clove oleoresin caused important lethal effect, *P. citrinum* and *A. niger* were more resistant. After 60 minutes, clove oleoresin dispersed (0.4% v/v) in concentrated sugar solution caused a 99.6% reduction of the initial population (10⁶ c.f.u./ml) of *Trichophyton mentagrophytes*. The fungicidal activity of clove-sugar on *C. albicans*, after 2 min contact, was similar to that presented by disinfectants commonly used in hospitals, such as povidone-iodine and chloroxylenol.

Key words: clove oil, fungicide, disinfectant, sugar

INTRODUCTION

In the past few years several papers have been published on the use of sugar (sucrose) for the treatment of infected wounds. These paper comprised clinical studies (10,11,14,17,20) and “*in vitro*” experiments on the antimicrobial activity of sucrose against bacteria pathogenic for humans (1,4,18). Although it has been well established that pathogenic bacteria cannot grow in sugar solutions with low water activity (a_w), the lethal effect of sugar on certain pathogens is slow and clinical situations may exist where the killing of these microorganisms in a short time is desirable. Previous attempts to increase the efficacy of sugar therapy were reported by Briozzo *et al.* (3) and Chirife *et al.* (6), who studied the “*in vitro*” antibacterial effect of clove oleoresin dispersed in a concentrated (saturated) sugar solution ($a_w = 0.83$). They reported that this system had a marked bactericidal effect comparable to that of various disinfectants which are commonly used in hospitals. They determined that clove oleoresin was responsible for the bactericidal action.

Although the water activity of a saturated sugar solution is low enough ($a_w = 0.83$) to inhibit the growth of pathogenic bacteria, some molds may be able to grow since their minimal water activity for growth is below 0.83 (19). The present work studied the effect of the addition of clove oleoresin (*Eugenia caryophyllata*) on the survival of several molds in saturated sugar solutions.

MATERIALS AND METHODS

Microorganisms

Candida albicans MN 84031, *Aspergillus niger* ATCC 16404, *Penicillium citrinum* F-300, and *Trichophyton mentagrophytes* ATCC 94795 were used in this study. The microorganisms were grown on malt extract agar (Merck, Darmstadt, Germany), except *T. mentagrophytes*, which was cultured in Sabouraud - glucose 2% agar (Merck, Darmstadt, Germany). *Candida albicans* was incubated for 24 h. at 37°C and the other three strains (*A. niger*, *P. citrinum* and *T. mentagrophytes*) were incubated for 15 days at

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25°C. After growth, *C. albicans* cells and spores of the fungal strains were suspended in saline solution with 0.05% Tween 80. The suspensions were filtered with sterilized gauze and adjusted to a concentration of approximately 10^8 cell/ml.

Test solution for antimicrobial activity

The test solution was prepared with refined granulated cane sugar (63% w/w), acquired from a retail store, to which variable amounts (0.2-0.8% v/v) of food-grade clove oleoresin (CO), provided by Fritzsche S.A.I.C.A. (Buenos Aires, Argentina), was added. The CO was dissolved in PEG-400 (50% v/v) (3).

The fungicidal effect of CO-sugar was compared with disinfectants commonly used in hospitals, such as povidone-iodine (1% of free iodine) at 0.5% and soapy solution of chloroxylenol (0.3%).

Fungicidal effect

Trials to determine the fungicidal effect were made following a previously described procedure (3,5). Briefly, 9.9 ml of test solution were placed in a water bath at 21°C or 37°C, and 0.1 ml of inocula was added. Samples were withdrawn periodically, diluted with the appropriate inactivator and submitted to microbial counts as described below. Plates were incubated at 25°C for 48 h for *C. albicans* and 5 days for the other microorganisms.

CO and p-chloroxylenol were inactivated using 3% Tween 80 and povidone-iodine with 0.1% sodium thiosulfate; the effectiveness of inactivation was experimentally evaluated following a previously described procedure (5).

All neutralized dilutions were surface-spread in petri dishes with malt extract agar (Merck, Darmstadt, Germany), except *T. mentagrophytes*, which was plated on Sabouraud - glucose 2% agar (Merck, Darmstadt, Germany).

Control Tests

The following set of controls were performed: (a) to determine whether or not sugar and PEG-400 possess antimicrobial activity; and (b) to determine whether a vehicle other than the sugar solution (distilled water) has influence on the antimicrobial activity.

(a) Antimicrobial activity of sugar and PEG- 400

Candida albicans or *A. niger* (0.1 ml) were added to 9.9 ml of sugar solution and to 9.9 ml of sugar solution containing 0.04 ml of PEG-400. The suspensions were thoroughly mixed and maintained at 21°C. After 2 min for the sugar solution and 10 min for the sugar solution with PEG-400, a sample was withdrawn and submitted to microbial count.

(b) Effect of a vehicle other than sugar solution

Candida albicans or *A. niger* (0.1 ml) were added to 9.9 ml of distilled water and to 9.9 ml of sugar solution containing 0.4% of clove oil. The suspensions were mixed and samples were withdrawn after 2 or 10 min at 21°C and counted.

RESULTS AND DISCUSSION

As we described in a previous study (4), clove oil caused rapid killing of *C. albicans*. This was confirmed in this study. Sugar solution or sugar solution with PEG-400 or concentrated sugar solution alone did not have the same lethal effect on microorganisms (4,18).

Similar antifungal properties of CO were observed both in distilled water and sugar solution (3). Nevertheless, the concentrated sugar solution provided a good vehicle in that a relatively more stable dispersion of the oil was easily obtained; this was not the case with distilled water since oil droplets formed soon after oil was dispersed.

Fig. 1 shows the effect of temperature (21°C or 37°C) and concentration of clove oleoresin (0.2 or 0.4% v/v) dispersed in the concentrated sugar solution on the survival of *Candida albicans* cells. Clove oleoresin had a fast killing effect on yeast cells and the lethal effect was higher at 37°C than at 21°C. The increase of concentration of CO from 0.2 to 0.4% produced a significant increase in the lethal action. At 37°C and 0.4% CO, a contact time of 1 min reduced drastically the inoculum from about 10^7 c.f.u./ml to 20 c.f.u./ml. At 21°C and 0.2% a biphasic behavior was observed instead of a simple logarithmic straight line. This result can be attributed to phenotypic and / or genotypic heterogeneity (8).

Figs. 2 and 3 show the effects of clove oleoresin concentration (0.4 and 0.8% v/v) and temperature (21°C and 37°C) on the survival of *Aspergillus niger* and *Penicillium citrinum*. The initial concentrations of inoculum in Fig. 2 were not the same because they represented different samples. The fungicidal effect of CO on molds was less evident than on *C. albicans*, and *A. niger* was much more resistant than *P. citrinum*. None of the survival curves presented a simple logarithmic straight line. Increase of temperature from 21°C to 37°C improved the effectiveness of CO towards both molds. Chirife *et al.* (5) also related increased bactericidal effect of

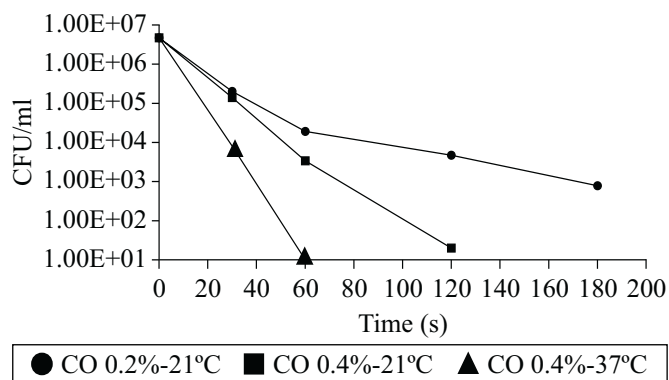


Figure 1. Effect of clove oleoresin (CO) dispersed in a concentrated sugar solution on *Candida albicans* at 21°C and 37°C.

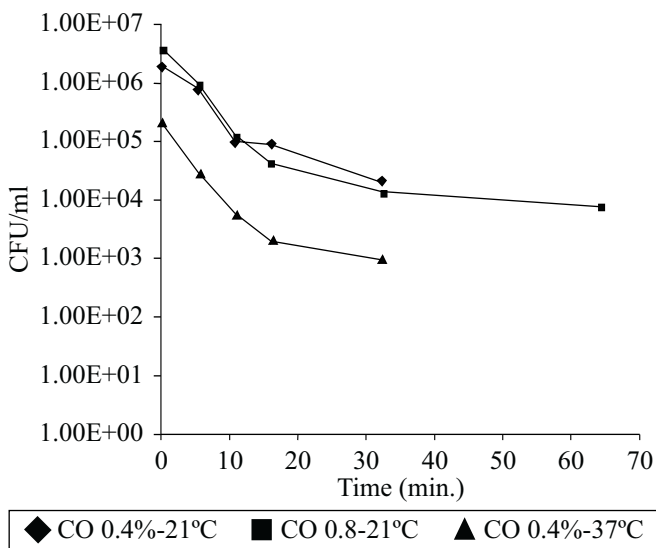


Figure 2. Effect of clove oleoresin (CO) dispersed in a concentrated sugar solution on *Aspergillus niger* at 21°C or 37°C.

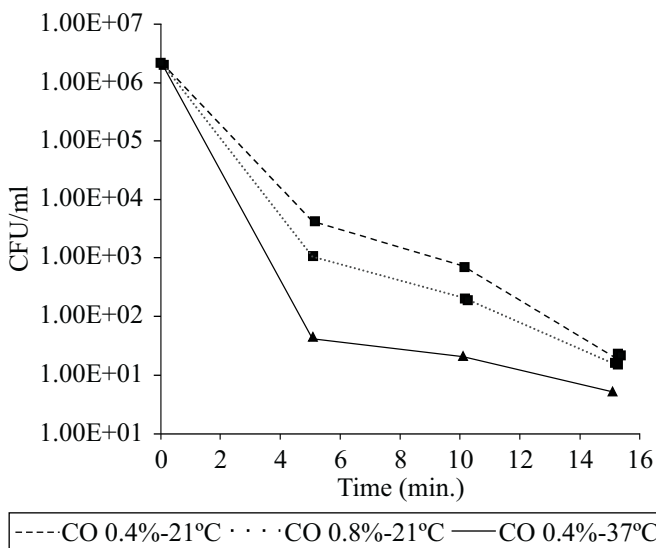


Figure 3. Effect of clove oleoresin (CO) dispersed in a concentrated sugar solution on *Penicillium citrinum* at 21°C or 37°C.

CO against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* when the temperature was raised from 21°C to 37°C. However, concentration had minor effect on the survival curves of *A. niger* and *P. citrinum* in the range studied (0.4 to 0.8%).

Despite the higher resistance of molds, clove oleoresin performed satisfactorily in disinfection since 99% (*A. niger*) and 99.99% (*P. citrinum*) of the exposed population were destroyed within 10 and 5 min contact time, respectively.

Table 1 compares the lethal effect of clove oleoresin and some disinfectants commonly used in hospitals, like povidone-iodine and chloroxylenol, on *C. albicans*. After 2 min at 21°C, CO presented the same “*in vitro*” killing effect as the disinfectants. However, for very short contact times (e.g. 1 min.), the later were more effective.

Fig. 4 shows the effect of CO dispersed in concentrated sugar solution on the survival curve of *Trichopyton mentagrophytes* NC 94797. After 60 min, a destruction of 99.6% of the 10^6 c.f.u./ml inoculum was observed.

Formerly it was assumed that the fungicidal action was due to clove oleoresin and not to the concentrated sugar solution, which was used mainly as a vehicle for the oleoresin (3). It is well known that concentrated sugar solution has a fungistatic rather than a fungicidal action (18) and this effect is usually explained by the decrease of water activity. Taking into account that the water activity of the sugar solution is 0.83 (4) and the minimal a_w for growth of *A. niger* and *P. citrinum* are 0.77 and 0.80 respectively

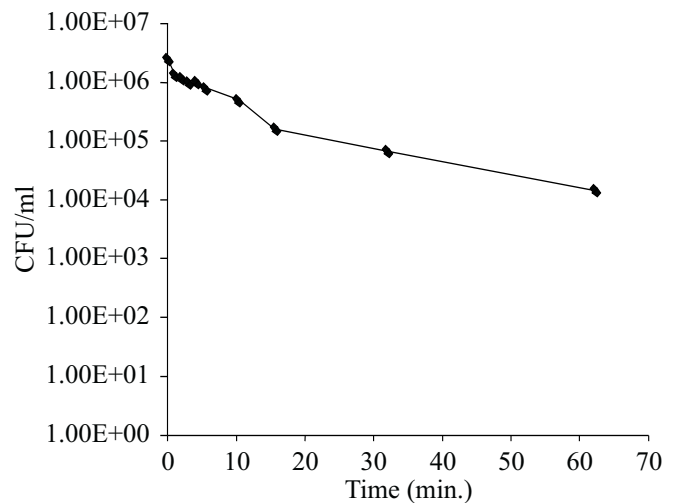


Figure 4. Effect of clove oleoresin (CO) dispersed in a concentrated sugar solution on *Trichophyton mentagrophytes* at 21°C.

Table 1. Effect of clove oleoresin (0.4% v/v) dispersed in concentrated sugar solution and disinfectants commonly used in hospitals (povidone-iodine and chloroxylenol) in *Candida albicans*.

Time of contact (sec)	Survivors, %		
	Clove (0.4%)	Povidone (0.5%)	Chloroxylenol (0.3%)
0	100	100	100
30	5	0.01	0.1
60	0.1	0.005	0.05
120	0.001	0.001	0.005
180	<0.001	<0.001	0.001

(16), a lower a_w should play only a minor role in the fast fungicidal action attributed to clove oleoresin. Selwyn and Durodie (18) studied the behavior of *C. albicans* in a saturated sugar solution, and reported growth inhibition followed by a slow reduction in number of cells as a function of contact time. A contact time of 12 hours was required to reduce the inoculum size by one log cycle. This contrasts with the very fast fungicidal action observed in present work for the clove oleoresin–sugar mixture.

Spices, herbs and their essential oils, commonly used as food seasonings, present well known antimicrobial activity (9,12,15). Benjilali *et al.* (2) reported the antifungal properties of essential oil of various plants (thyme, rosemary, mugwort and various species of mint) which were tested against 39 mold strains. Mahmoud (13) tested the antifungal effect of some essential oils on growth of molds and aflatoxin production. Clove was among the substances with antifungal activity. Connor and Beuchat (7) observed that 32 different essential oils from plants, allspice, cinnamon, clove, garlic, oregano, savoury, and thyme were particularly inhibitory to selected food spoilage and industrial yeasts.

Results of the present work demonstrated that the association of clove oleoresin with concentrated sugar has a strong fungicidal effect. Further studies are required to demonstrate the usefulness of this association for topical treatment of mycotic infections. Nevertheless, absence of any harmful effect on human tissues should be demonstrated before clove oleoresin is recommended for clinical application. No adverse effect to normal wound healing or any other unfavorable effect should also be demonstrated.

RESUMO

Propriedades antifúngicas do óleo de cravo (*Eugenia caryophyllata*) numa solução de açúcar

No estudo avaliou-se o efeito da temperatura, concentração e tempo de contato na atividade antifúngica do óleo essencial do cravo disperso em solução concentrada de açúcar. Os ensaios foram feitos a 21°C e a 37°C, utilizando suspensão de óleo essencial de 0,2 a 0,8% v/v. Os microorganismos utilizados foram *Candida albicans*, *Penicillium citrinum*, *Aspergillus niger* e *Trichophyton mentagrophytes*. A melhor atividade fungicida ocorreu a 37°C, onde um minuto de contato foi suficiente para matar uma população de 10^6 u.f.c./ml de *Candida albicans*. Não obstante, *P. citrinum*, *A. niger*, *T. mentagrophytes* foram mais resistentes, embora uma importante ação letal da essência tenha sido observada. Em 60 minutos, o óleo essencial de cravo (0,4% v.v.) em solução concentrada de açúcar reduziu em 99,6% um inóculo de 10^6 u.f.c./ml de *T. mentagrophytes*. O efeito letal do óleo essencial de cravo sobre *C. albicans*, após 2 minutos de contato, foi semelhante ao de desinfetantes comumente usados em hospitais, como povidona-iodo e cloroxilenol.

Palavras-chave: cravo, fungicida, desinfetante, açúcar

REFERENCES

- Ambrose, U.; Middleton, K.; Seal, D. *In vitro* studies of water activity and bacterial growth inhibition of sucrose- polyethylene glycol- 400- hydrogen peroxide and xylose-polyethylene glycol-400-hydrogen peroxide past used to treat infected wounds. *Antim. Agents and Chemoth.*, 35:1799-1803, 1991.
- Benjilali, B.; Tantaoui-Elaraki, A.; Ayadi, A.; Ihlal, M. Method to study antimicrobial effects of essential oils: application to the antifungal activity of six Moroccan essences. *J. Food Protec.*, 47: 748-752, 1984.
- Briozzo, J.; Núñez, L.; Chirife, J.; Herszage, L.; D'Aquino, M. Antimicrobial activity of clove oil dispersed in a concentrated sugar solution. *J. Appl. Bacteriol.*, 66: 69-77, 1988.
- Chirife, J.; Herszage, L.; Joseph, A.; Kohn, E.S. *In vitro* study of bacterial growth inhibition in concentrated sugar solutions: microbiological basis for the use of sugar in the treatment of infected wound. *Antim. Agents Chemoth.*, 23: 766-773, 1983.
- Chirife, J.; Núñez, L.; Ballesteros, S.A.; Bozzini, J.P.; Herszage, L.; D'Aquino, M. Estudios sobre la acción bactericida del aceite de clavo de olor dispersado en una solución de azúcar. *Rev. Arg. Microbiol.*, 24: 32-39, 1992.
- Chirife, J.; Herszage, L.; Joseph, A.; Kohn, E.S. Effect of potassium sorbate and pH on the survival of *Staphylococcus aureus* in saturated sugar solution. *Rev. Arg. Microbiol.*, 25:80-87,1993.
- Conner, D.E.; Beuchat, L. Sensitivity of heat-stressed yeast to essential oils of plants. *Appl. Environm. Microbiol.*, 47: 229-233, 1984.
- D'Aquino, M.; Santini, P.; Muzio, H. Action of halogenated compounds on *Aspergillus conidiospores*. *J. Food Protec.*, 49: 537-540, 1986.
- Deans, S.G.; Ritchie, G. Antibacterial properties of plant essential oils. *Int. J. Food Microbiol.*, 5: 165-180, 1987.
- Gordon, H.; Middleton, K.; Seal, D.; Sullens, K. Sugar and wound healing. *The Lancet*, ii:663-664, 1985.
- Herszage, L.; Montenegro, J.R.; Joseph, A.L. Tratamiento de las heridas supuradas con azúcar granulado comercial. *Boletín de Trabajos de la Sociedad Argentina de Cirugía*, 41:315-330, 1980.
- Hili, P.; Evans, C.S.; Veness, R.G. Antimicrobial action of essential oils: the effect of dimethylsulphoxide on the activity of cinnamon oil. *Lett. Appl. Microbiol.*, 24:269-275, 1997.
- Mahoud, L.E. Antifungal action and antiaflatoxicogenic properties of some essential oil constituents. *Lett. Appl. Microbiol.*, 19:110-113, 1994.
- Middleton, K.R.; Seal, D. Sugar as an aid to wound healing. *Pharmaceutical J.*, 235:757-758, 1985.
- Morris, J.A.; Khettry, A.; Seitz, E.W. Antimicrobial activity of aroma chemicals and essential oils. *J. Amer. Oil Chemists' Soc.*, 56:595-603, 1979.
- Pitt, J.I. Food spoilage by xerophilic fungi. In: "Water Relations of Food". Ed. by R.B. Duckworth, Academic Press, New York, 1974, p.270-284.
- Seal, D.V.; Middleton, K.R. Healing of cavity wounds with sugar. *The Lancet*, 338:571-572, 1991.
- Selwyn, D.; Durodie, J. The antimicrobial activity of sugar against pathogens of wounds and other infections of man. In: Simatos, D.; Multon, J.; Dordrech, (eds). *Properties of Water in Foods in relation to Quality and Stability*. The Netherlands, Martinus Nijhoff Pub., 1985, p.293-307.
- Troller, J.; Christian, J.H.B. Water Activity and Food. Academic Press, New York, 1978, p.28-34.
- Trouillet, J.L.; Fagon, J.Y.; Domart, Y.; Chastre, J.; Pierre, J.; Gibert, C. Use of granulated sugar in treatment of open mediastinitis after cardiac surgery. *The Lancet*, ii: 180-184, 1985.