Bactericidal Activity against *Vibrio Cholerae* of Chemical Products Used in Lemon Production in Tucumán, Argentina

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The present research was set up to verify whether the chemical products used in lemon production (from cultivation until packaging) have a bactericidal or a bacteriostatic activity against *Vibrio cholerae* O1. The studied products were: copper oxychloride, benomil (a carbamate), active chlorine, sodium-o-phenylphenoxoate, guazatine (a polyamine mixture), imazalil (an imidazole) and lemon peel. The latter was studied with and without treatment using the above mentioned chemicals. Different dilutions of these products were tried out with varying exposure times against the bacterium *V. cholerae* Serogroup O1, Biotype El Tor, Serotype Inaba. The concentrations of the microorganism ranged from $10^2$ to $10^6$ CFU ml$^{-1}$, the latter one being considered an infectious dose. The following results were obtained: 1) active chlorine (chlorinated water) showed bactericidal activity at concentrations of 50, 100 and 200 ppm after 10 min of exposure time, 2) copper oxychloride, sodium-o-phenylphenoxoate, guazatine and imazalil showed bactericidal activity against *V. cholerae* at concentrations of $10^2$ and $10^4$ CFU ml$^{-1}$, 3) due to the fact that during its cultivation the fruit is successively sprayed with several chemical products, it could be that the result of the successive treatments is superior to the result of a repeated treatment with each of the individual products. This consideration should be taken into account when evaluating the eventual protection of the lemon.

**Key words** *Vibrio cholerae*; lemon industry chemical; bactericidal; bacteriostatic

Cholera is one of the oldest diseases of humanity. At the end of 1991 an epidemic burst out in Peru, then expanded rapidly to many other Latin-American countries.1,2) We know that food is an important vector in the transmission of this disease which occurs immediately after direct contamination with human faeces, contact with sick people or contaminated drinking water in order of importance.3) Because Tucumán province is the number one lemon exporter in Argentina and the fourth in the world, we began this research with the aim to understand the survival of *V. cholerae* with respect to the different chemical products applied in the lemon industry: from cultivation until packaging. This study was realized in collaboration with the Tucumán, Citrus Association.

**MATERIALS AND METHODS**

**Studied Chemical Products.** 1. Copper oxychloride, 2. active chlorine (chlorinated water), 3. methyl 1-(butylcarbamoyl)-benzimidazol-2-buty carbamate, 50% (hereafter referred to as benomil, the general name), 4. sodium-o-phenylphenoxide, 5. a mixture of reaction products of polyamines, comprised mainly of octamethylene diamine, iminodi(octamethylene)diamine, octamethylenebis(mimo-octamethylene)diamine and carbazaminitrile, 40% (hereafter referred to as guazatine) and 6. (±)-1-(β-alloyxy-2,4-dichlorophenylethyl)mimidazole, 80% (hereafter referred to as imazalil). All products were purchased from the Tucumán Citrus Association. Different dilutions of all products mentioned were used against the bacterium *V. cholerae* Serogroup O1, Biotype El Tor, Serotype Inaba (toxin negative). The microorganism concentrations used were $10^2$, $10^4$, $10^6$ and $10^8$ CFU ml$^{-1}$, the latter being considered an infectious dose. Copper oxychloride, benomil, guazatine and imazalil were used in dilutions of $10^{-5}$, $10^{-6}$ and $10^{-7}$. The exposure time with *V. cholerae* was: 1, 3, 10 and 24 h. Active chlorine was used in concentrations of 50, 100 and 200 ppm. The exposure time was: 0.5, 1, 10 and 30 min. Sodium-o-phenylphenoxoate was used in dilutions of $10^{-1}$, $10^{-2}$ and $10^{-3}$. The exposure time was: 10, 30 and 60 min.

**RESULTS AND DISCUSSION**

The following results were obtained: 1) Active chlorine (Fig. 1) showed bactericidal activity at concentrations of 50, 100 and 200 ppm after 10 min of exposure time. 2) Copper oxychloride (Fig. 2), sodium-o-phenylphenoxoate (Fig. 3), imazalil (Fig. 4) and benomil (Fig. 5) showed bactericidal activity against *V. cholerae* at concentrations of $10^2$ and $10^4$ CFU ml$^{-1}$. 3) All guazatine dilutions assayed (Fig. 6) inhibited *V. cholerae* at a concentration of $10^2$ CFU ml$^{-1}$. For concentrations of the microorganism of $10^6$ and $10^8$ CFU ml$^{-1}$, only partial inhibition of *V. cholerae* was observed.

Halstead,6) McIntyre et al.5) and Yan et al.6) showed that *V. cholerae* is able to survive for long periods under extreme conditions such as low temperatures and a reduced concentration of nutrients. Mossel et al.7) suggest that a technological level of food safety should be implemented, which would assure a good economic benefit to exporting countries. This can be obtained with a rigorous application of preventive sanitary measures that permit a guarantee of the microbiological quality of all the exported products. This result coincides with observations made by Felsenfeld,8) who studied the effect of chlorinated solutions and a permanganate solution on food products and concluded that the chlorinated solutions are more effective under well controlled conditions of time and concentration.

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Fig. 1. Bactericidal Activity of Active Chlorine at a Concentration of 100 ppm against *V. cholerae* at Concentrations of 10^2 (■), 10^4 (+), 10^6 (▲) and 10^8 (□) CFU ml⁻¹

Fig. 2. Bactericidal Activity of Copper Oxchloride (Dilution 10⁻⁶) against *V. cholerae* at Concentrations of 10^2 (■), 10^4 (+), 10^6 (▲) and 10^8 (□) CFU ml⁻¹

Fig. 3. Bactericidal Activity of Sodium-o-phenylenediamine (Dilution 10⁻²) against *V. cholerae* at Concentrations of 10^2 (■), 10^4 (+), 10^6 (▲) and 10^8 (□) CFU ml⁻¹

Fig. 4. Bactericidal Activity of Imazalil (Dilution 10⁻⁶) against *V. cholerae* at Concentrations of 10^2 (■), 10^4 (+), 10^6 (▲) and 10^8 (□) CFU ml⁻¹

Fig. 5. Bactericidal Activity of Benomil (Dilution 10⁻⁶) against *V. cholerae* at Concentrations of 10^2 (■), 10^4 (+), 10^6 (▲) and 10^8 (□) CFU ml⁻¹

Fig. 6. Bactericidal Activity of Guazatine (Dilution 10⁻⁶) against *V. cholerae* at Concentrations of 10^2 (■), 10^4 (+), 10^6 (▲) and 10^8 (□) CFU ml⁻¹

With regard to our results, and due to fact that the fruit is successively sprayed with several chemical products during the industrial process, including its cultivation, it can be postulated that the sum of the effects of each of the individual products leads to a final treatment which is even more secure.

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REFERENCES
