EFFECTS OF THYME (ZAATAR) ESSENTIAL OIL AND SOME CHEMICAL COMPOUNDS IN THE CONTROL OF CITRUS BACTERIAL CANKER IN IRAN

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SUMMARY

The efficiency of two novel compounds, thyme essential oil (TEO) produced from zaatar (*Zataria multiflora*) and Nanosilver (NS), as well as some commonly used chemicals were evaluated against citrus bacterial canker using detached-leaf assays in the laboratory and whole seedlings in the greenhouse. Mexican lime seedlings were used for detached leaf assays. Treatments included 0.3% copper oxychloride (COC), 1.5% Bordeaux mixture (BO), 0.3% COC+0.04% Mancozeb (MZ), 1.5% BO+0.04% MZ, TEO at 10^{-2} dilution, three concentrations of NS (100, 150 and 200 ppm) and 100 ppm Streptomycin (S). The BO+MZ, COC+MZ and TEO treatments were found most effective based on mean number of lesions and type of symptoms on detached leaves. Greenhouse experiments were done to assess the protective effects of the same treatments. Nine-month-old seedlings were sprayed with the treatments and after 24h, the young leaves were inoculated with bacterial suspension by pin-prick. The number of lesions on treated leaves was counted 14 days after inoculation and rates of infection were measured. Results showed that all treatments were significantly different at $P<0.01\%$. The best results were obtained with BO+MZ and TEO at 10^{-2} dilution, with infection inhibition of 78.44% and 69.78%, respectively. Moreover, COC+MZ, BO, S and COC alone reduced the disease by 60.15%, 55.15%, 45.78%, and 41.09%, respectively. The least effective compound was 100 ppm NS which only reduced the infection by 15%.

Key words: Xanthomonas citri pv. *citri* (Hasse)*, Zataria multiflora*, Nanosilver, Copper compounds, control.

INTRODUCTION

Citrus bacterial canker (CBC), caused by *Xanthomonas citri* pv. *citri* (Hasse) (*Xcc*) is a widespread disease in citrus-producing areas of the tropics and subtropics. It is a serious disease of most commercial citrus cultivars and species (Civerolo, 1984).

Crater-like lesions with a raised margin and sunken centre surrounded by a yellow halo characterize the disease (Graham *et al*., 2004). Severe infection produces a variety of effects including defoliation, dieback, severely blemished fruit, reduced fruit quality and premature fruit drop. Warm, humid, cloudy weather, along with heavy rainfall and strong wind promote the disease. Control in countries or regions where CBC is not present include quarantine or regulatory programmes that prohibit introduction of infected citrus plant material and fruit, as well as continuous and strict surveying in the field and immediate destruction of infected trees. In countries where canker is present, integrated systems of compatible cultural practices and phytosanitary measures consisting of resistant hosts, removal of inoculum sources, properly designed windbreak systems, timely application of protective copper-containing and antibiotic sprays are generally the most effective means of disease management (Das, 2003). Using copper-based bactericides is a standard method to control CBC worldwide (Koizumi, 1985; Leite and Mohan, 1990).

Copper bactericides have possible disadvantages after long-term use, including resistance to copper in Xanthomonad populations as well as accumulation of copper in soils with potential phytotoxicity and environmental effects (Alva *et al*., 1995). Antibiotics are not as effective as copper-based products (Leite and Mohan, 1990; Timmer, 1988) and there is also some evidence of resistance development within bacterial populations (Ritchie and Dittspongpitch, 1991).

Silver compounds have long been recognized for their wide range of antimicrobial activities. Some researchers suggest that silver nanoparticles damage bacterial cells by penetrating the cell wall, subsequently turning DNA into a condensed form and loosing its replicating ability, interacting with thiol groups and inactivating the enzymes activity, weakening the plasma membrane or the cell wall as well as by deposition of some proteins in cells (Feng *et al*., 2000). A study on *Escherichia coli* showed that Nanosilver damages and pitts bacterial cell walls in which it accumulates, leading to increased cell permeability and, ultimately, to cell death. *E. coli* is often used as a model for gram negative bacteria, suggesting that these results could have a broader

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relevance (Sondi and Salopek-Sondi, 2004). The microbicidal effects involve both altering the function of the cell membrane and linking to the cell DNA, disrupting cell reproduction (Russell and Hugo, 1994).

Plant essential oils and extracts are of interest as an alternative source of natural pesticide for the control of plant pests and diseases (Reddy and Rao, 1960; Boonywanich and Panutat, 1998; Satish *et al*., 1999; Leksomboon *et al*., 2000, 2001; Omidbeygi *et al*., 2007). *Zataria multiflora* (Boiss) (Avishan-e-Shirazi in Persian and Sa'atar or Zaatar in the old Iranian medical books), a kind of thyme (Labiatae), grows naturally in the central and southern parts of Iran (Amin, 1991). Its essential oil was found to contain 26 different substances such as thymol (48.4%) and carvacrol (12.6%) which are antimicrobial and antifungal (Shafiee and Javidnia, 1997).

This study was done with the aim of finding a suitable method of using thyme essential oil (TEO) as an alternative to chemical control of CBC.

MATERIALS AND METHODS

Bacterial cultures. Forty eight strains of *Xcc* were isolated from citrus orchards in south Iran. A mixture including three representative strains, HBL3, RS6 and HA6 in equal proportions was used in this study. Prior to inoculation, bacteria were grown on nutrient agar (NA) at 28°C for 48 h. For inoculation, bacterial suspensions were prepared in sterile distilled water and spectrophotometrically adjusted to 10^8 CFU/ml^{-1} (OD 0.5 at 620 nm).

Chemical compounds. Chemicals used in this study included 0.3% copper oxychloride (COC), 1.5% Bordeaux mixture (BO), 0.3% COC+0.04% Mancozeb (MZ) (manufactured by Bahavar Shimi Co. Iran), 1.5% BO+0.04% MZ, three concentrations (100, 150 and 200 ppm) of Nanosilver [NS (Nanocide®, by Nano Nasb Pars Co. Iran)] and 100 ppm Streptomycin (S). The above concentrations remained invariant for all treatments.

Preparation of TEO dilutions. Standard TEO (of *Z. multiflora*) was obtained from Barij Essence Pharmaceutical Company, Kashan, Iran. A mixture containing pure thyme, Soprofor emulsifier (Soprofor FL 2.00®) and sterile distilled water at ratio 3:1:5 was used as stock. Three successive dilutions (10^{-1}, 10^{-2}, 10^{-3}) were then made.

Detached leaf assay. Immature fully expanded Mexican lime (*Citrus aurantifolia*) leaves from greenhouse-grown plants were washed for 10 min in running tap water, surface-sterilized in 1% sodium hypochlorite for 1-2 min and aseptically rinsed thoroughly with sterile distilled water. The leaves were sprayed with TEO as well as the other compounds. Five to six hours after spraying, four areas of about 0.5 cm^2 on the lower surface of each leaf were punctured with a sterile needle. Each wounded leaf was placed on 1% water agar in a Petri dish, with the lower surface up. Droplets (10 µl) of the bacterial suspension were placed on each of the wounded areas. Host reactions were recorded based on the number of lesions and type of symptoms 5-7 days after incubation at 25-30°C (Graham and Gottwald, 1990; OEPP/EPPO, 2005).

Greenhouse tests. Seeds of Mexican lime were sown in 6-litre pots and seedlings were fertilized weekly with Agroleaf 20+20+20® (Scotts Co, The Netherlands). Nine-month-old seedlings with uniform vigour and stem diameter (approximately 1 cm) were cut back to allow one dominant shoot to develop. When shoots had three to four almost fully expanded leaves, each of four plants was sprayed with *ca.* 20±5 ml of one of the treatments. Twenty-four hours after spraying, four leaves per plant as well as four areas per leaf were selected for puncturing. In each area, 20 wounds were made and the upper leaf surfaces immediately sprayed with mixture of *Xcc* strains. Inoculated seedlings were immediately covered with plastic bags for 48 h and were maintained in the greenhouse at 28-30°C with 80-90% relative humidity. After 14 days, numbers of lesions on treated leaves were counted using a X10 magnifier (Graham and Leite, 2004). The experiment was carried out in completely randomized design with 11 treatments and 4 replicates. One of the treatments including four seedlings inoculated with bacterial suspension as positive control. Four seedlings were sprayed only with sterile distilled water as negative control. The mean number of lesions observed in each treatment were analysed statistically (Table 2). Infection rate was measured by dividing mean lesion number in each treatment by number of wounds on leaves produced by needle puncturing (80 wounds) as below:

$$RI(\%) = LN/NW \times 100$$

where: RI=Infection Rate, LN=Mean number of lesions in each treatment, NW=Number of wounds on leaves (80 wounds).

Another index was infection inhibition which was estimated through the following function:

$$II(\%) = (RI_C\ \% - RI_I\ \%)$$

where: II=Infection inhibition, RI_C=Infection rate in positive control treatment (C+), RI_I=Infection rate in expected treatment.

Statistical analysis. Data were worked out by analysis of variance (ANOVA) and differences among the means were determined for significance at P<0.01 using Duncan's multiple range test (MSTATC software).

Table 1. Effects of chemical compounds and essential oil of thyme (*Zataria multiflora*) on *Xanthomonas citri* pv. *citri* in detached leaf assay.

Treatment	Number of lesions[a]	Type of symptoms	Rate of Control
0.3% copper oxychloride+0.04% Mancozeb 1.5% Bordeaux mixture+0.04% Mancozeb Thyme essential oil (stock diluted 10^{-2})	<10	Necrotic tissue and limited soft lesions	Effective
0.3% copper oxychloride 1.5% Bordeaux mixture 100 ppm Streptomycin	10-15	Moderate symptoms with superficial lesions	Mild
100 ppm Nanosilver 150 ppm Nanosilver 200 ppm Nanosilver Positive control (C+)[b]	>15	Friable, callus-like, whitish tissue	Ineffective
Negative control (C-)[c]	0	No symptoms	No control

[a]Mean number of lesions per detached leaf . [b]Bacterial suspension only. [c]Sterile distilled water only.

RESULTS

Detached leaf assay. Treatments were ranked in three groups based on the mean number of lesions observed in each leaf. The most efficient compounds were BO+MZ, COC+MZ and TEO at 10^{-2} dilution which decreased the mean number of lesions <10 on detached leaves, whereas the undiluted stock solution of TEO as well as the 10^{-1} dilution caused severe phytotoxicity. Ten to fifteen lesions appeared after treatments with COC, BO and S. Symptoms, i.e. superficial lesions, were moderate. Application of NS (100, 150 and 200 ppm) was not effective in reducing lesions (>15) on detached leaves as compared to the positive control showing friable, callus-like and whitish tissue symptoms. No symptoms and lesions were observed in negative control treatment (Table 1).

Greenhouse tests. Results showed a significant difference among all treatments at $P<0.01$ on the number of lesions observed in each leaf. According to the Duncan multiple range test, treatments fell into seven groups ("a" to "g " in Table 2 and Fig. 1). The lowest number of lesion was observed in the negative control (C-) followed by BO+MZ (14.50) and TEO (21.50). These two latter treatments were in the same statistical group (f). On the other hand, as expected, the highest lesion numbers were observed in the positive control (C+) (77.25) and NS 100 ppm (65.25). The mean lesion numbers in the following treatments were not significantly different ($P<0.01$): NS 150 ppm (57.50) and NS 200 ppm (54.38); COC (44.38) and S (40.63); BO (33.13) and COC+MZ (29.13). The results also showed that the highest inhibition of infection was afforded by BO+MZ (78.44%) and TEO (69.78%), suggesting that these compounds could be the most effective in the control of CBC. Furthermore, COC+MZ, BO, S, COC, NS 200 ppm, NS 150 ppm, and NS 100 ppm reduced disease incidence by 60.15%, 55.15%, 45.78%, 41.09%, 28.59%, 24.69% and 15%, respectively. Generally, the greenhouse results confirmed those of detached leaf assays. The best results on controlling of disease were observed in BO+MZ and TEO treatments in both conditions (Tables 2 and 3).

DISCUSSION

In the detached leaf assay, the most effective compounds in decreasing symptoms of bacterial canker were BO+MZ, COC+MZ and TEO at 10^{-2} dilution. Undiluted and 10^{-1} dilution of the stock preparation of TEO showed severe phytotoxicity on detached leaves. Mild control and moderate symptoms with superficial lesions were observed after treatments with COC, BO and S. These treatments reduced the number of lesions by 85 to 90%. Typical symptoms of citrus canker such

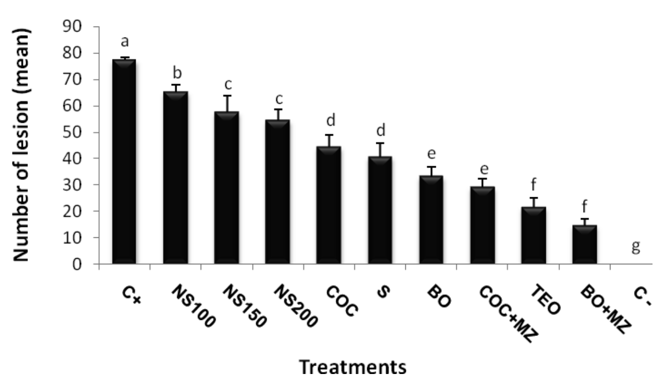

Fig. 1. Effects of different treatments on means of lesion numbers in greenhouse experiment. C +: Positive control; NS 100: 100 ppm Nanosilver; NS150: 150 ppm Nanosilver; NS200: 200ppm Nanosilver; COC: 0.3% copper oxychloride; S: 100 ppm Streptomycin; BO: 1.5% Bordeaux mixture; COC+MZ: 0.3% Copper oxychloride+0.04% Mancozeb; TEO: Thyme essential oil, stock diluted 10^{-2}; BO+MZ: 1.5% Bordeaux mixture +0.04% Mancozeb; C -: Negative control.

Table 2. Means of lesion numbers in each replicate (greenhouse experiment).

	Replicates				
Treatment	I	II	III	IV	Mean
0.3% Copper oxychloride	42.00	40.25	45.00	50.25	44.38d
1.5% Bordeaux mixture	35.00	31.25	37.25	29.00	33.13e
0.3% Copper oxychloride+0.04%Mancozeb	28.75	25.75	33.50	28.25	29.13e
1.5% Bordeaux mixture+0.04% Mancozeb	14.75	12.25	18.00	13,00	14.50f
Thyme essential oil (stock diluted 10^{-2})	25.50	18.00	19.00	23.50	21.50f
100 ppm Streptomycin	43.00	45.75	33.75	40.00	40.63d
100 ppm Nanosilver	61.75	64.50	66.75	68.00	65.25b
150 ppm Nanosilver	58.50	66.00	51.00	54.50	57.50c
200 ppm Nanosilver	58,25	54.50	48.75	56.00	54.38c
Positive control (C+)	76.00	77.25	78.25	77.50	77.25a
Negative control (C-)	00.000	00.00	00.00	00.00	00.00g

Figures followed by the same letter are not significant at $P<0.01$

Table 3. Effects of chemical compounds and thyme essential oil on inhibition of citrus bacterial canker under greenhouse conditions.

Treatment	Infection rate (%)	Infection inhibition (%)
0.3% copper oxychloride	55.47	41.09
1.5% Bordeaux mixture	41.41	55.15
0.3% copper oxychloride + 0.04% Mancozeb	36.41	60.15
1.5% Bordeaux mixture + 0.04% Mancozeb	18.12	78.44
Thyme essential oil (stock diluted 10^{-2})	26.87	69.78
100 ppm Streptomycin	50.78	45.78
100 ppm Nanosilver	81.56	15.00
150 ppm Nanosilver	71.87	24.69
200 ppm Nanosilver	67.97	28.59
Positive control (C+)	96.56	0.00
Negative control (C-)	0.00	-

as friable, callus-like as well as whitish tissue could be observed using Nanosilver (100, 150 and 200ppm) and in the positive control. In greenhouse experiments all compounds significantly decreased ($P<0.01$) the number of lesions. The best results were obtained with BO+MZ and TEO, which reduced disease by 78.44% and 69.78%, respectively.

Copper compounds are recommended for CBC control. Results showed that using a mixture of COC and BO with MZ reduced disease 19.06% and 23.29% more than using COC and BO alone, respectively (Table 3). Apart from better disease control, to avoid copper resistance, it is appropriate to use copper-containing compounds in mixture with Mancozeb (Canteros, 2000). Integrated control strategies have also been applied. For example, pruning CBC-affected twigs every year in November-December followed by 3 to 4 sprays of 1% Bordeaux mixture a year reduced the disease (Patel and Desai, 1970), and similar positive effects were reported by Kishun and Chand (1987) with two prunings accompanied by 4 sprays of 5000 ppm copper oxychloride or 1% Bordeaux mixture.

According to Rangaswami *et al.* (1959), 500-1000 ppm Streptomycin sulphate was effective when sprayed with 1% glycerine on lime. Six sprays of 1000 ppm Streptomycin sulphate along with two prunings reduced bacterial canker in acid lime (Balaraman and Purushotman, 1981). Other effective antibiotics were Agrimycin (Sawant *et al.*, 1985), Streptocycline (Mathur *et al.*, 1973) and Streptocycline in combination with Bordeaux mixture (Krishna and Nema, 1983). In field trials with 7 different chemicals, the best control of *Xcc* was obtained using Paushamycin+Blitox followed by Bordeaux mixture (Kale *et al.*, 1988). Kale *et al.* (1994) also suggested that for better control of canker, spraying of Streptocycline+copper oxychloride (0.1%) should preferably be done at 7 or 15 day intervals. Integrated pruning of infected twigs and application of 0.3% copper oxychloride, 100ppm Streptocycline and neem cake suspension was found very effective in controlling CBC (Das and Singh, 2000).

There are several reports that confirm TEO inhibition of some phytopathogenic microorganisms. For example, the antifungal activity of TEO was shown against

Aspergillus flavus (Omidbeygi *et al*., 2007). Extracts from *Tamarindus indica* (Tamarind) were the most effective in controlling CBC in greenhouse and field experiments (Leksomboon *et al*., 2001). Also, the growth of *Xcc* and *Xanthomonas oryzae* was inhibited by extracts from seeds of *Hydnocarpus anthelmintica* (Boonywanich and Panutat, 1998). Application of neem cake solution to the foliage reduced CBC in nurseries (Reddy and Rao, 1960).

Nanosilver as a second novel compound did not show the expected results in this study. Nanosilver 100 ppm with 81.56% infection rate was the least effective compound. Our studies indicated that BO+MZ, as well as TEO, could be effective in limiting the disease. TEO can be used by alternating spray with copper and other chemical compounds.

The effect of TEO on CBC has not previously been reported. In any case, further studies on TEO phytotoxicity and environmental impact on epiphytic beneficial microorganisms are needed before any practical application.

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REFERENCES

Alva A.K., Graham J.H., Anderson C.A., 1995. Soil pH and copper effects on young Hamlin orange trees. *Soil Science Society of America Journal* **59**: 481-487.

Amin G.H., 1991. Popular Medicinal Plants of Iran. Deputy Minister of Research Publication, Ministry of Health, Treatment and Medical Education, Tehran, Iran. **1:**40.

Balaraman K., Purushotman R., 1981. Control of citrus canker on acid lime. *South Indian Horticulture* **29**: 175-177.

Boonywanich S., Panutat P., 1998. Studies on antibacterial potential of extracts from *Hydnocarpus anthelmintica* against plant pathogenic bacteria. Science and Technology Publishing House, Bangkok, Thailand.

Canteros B.I., 2000. Citrus canker in Argentina: control, eradication and current management. *Proceedings International Citrus Canker Research Workshop*, *Fort Pierce, 2000*: 10-11.

Civerolo E.L., 1984. Bacterial canker disease of citrus. *Journal of the Rio Grande Valley* **37**: 127-146.

Das A.K., 2003. Citrus canker, a review. *Journal of Applied Horticulture* **5**: 52-60.

Das A.K., Singh S., 2000. Management of acid lime canker by using chemicals with compatible cultural practices. Hi Tech Citrus Management. *Proceedings of the International Symposium of Citriculture*, *Nagpur 1999*: 1054-1056.

Feng Q.L., Wu J., Chen G.Q., Cui F.Z., Kim T.N., Kim J.O., 2000. A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. *Journal of Biomedical Material Research* **52**(4): 662-668.

Graham J.H., Gottwald T.R., 1990. Variation in aggressiveness of *Xanthomonas campestris* pv. *citrumelo* associated with citrus bacterial spot in Florida citrus nurseries. *Phytopathology* **80**: 190-196.

Graham J.H., Gottwald T.R., Cubero J., Achor D.S., 2004. *Xanthomonas axonopodis* pv. *citri*: factors affecting successful eradication of citrus canker. *Molecular Plant Pathology* **5**: 1-15.

Graham J.H., Leite R.P., 2004. Lack of control of citrus canker by induced systemic resistance compounds. *Plant Disease* **88**: 745-750.

Kale K.B., Kolte S.O., Peshney N.L., 1994. Economics of chemical control of citrus canker caused by *Xanthomonas campestris* pv *citri* under field conditions. *Indian Phytopathology* **47**: 253-255.

Kale K.B., Raut J.G., Ojekar G.B., 1988. Efficacy of fungicides and antibiotics against acid lime canker. *Pesticides* **22**: 26-27.

Kishun R., Chand J.N., 1987. Studies on germplasm resistance and chemical control of citrus canker. *Indian Journal of Horticulture* **44**: 126-132.

Koizumi M., 1985., Citrus canker: The world situation. *In:* Timmer L.W. (ed). Citrus Canker: An International Perspective, pp. 2-7. Citrus Research and Education Center, University of Florida, Lake Alfred, FL, USA.

Krishna A., Nema A.G., 1983. Evaluation of chemicals for the control of citrus canker. *Indian Phytopathology* **36**: 348-350.

Leite R.P., Mohan S.K., 1990. Integrated management of the citrus bacterial canker disease caused by *Xanthomonas campestris* pv. *citri* in the State of Paraná, Brazil. *Crop Protection* **9**: 3-7.

Leksomboon C., Thaveechai N., Kositratana W., Paisooksantivatana Y., 2000. Antiphytobacterial activity of medicinal plant extracts, *Science* **54**: 91-97.

Leksomboon C., Thaveechai N., Kositratana W., 2001. Potential of plant extract for controlling citrus canker of lime. *Kasetsart Journal of Natural Sciences* **32**: 392-396.

Mathur A.S., Irulappan I., Godhar R.B., 1973. Efficacy of different fungicides and antibiotics in the control of citrus canker caused by *Xanthomonas citri* (Hasse) Dowson. *Mysore Agricultural Journal* **60**: 626.

OEPP/EPPO, 2005. *Xanthomonas axonopodis* pv. *citri*. *Bulletin OEPP/EPPO Bulletin* **35**: 289-294.

Omidbeygi M., Barzegar M., Hamidi Z., Naghdibadi H., 2007. Antifungal activity of thyme, summer savory and clove essential oils against *Aspergillus flavus* in liquid medium and tomato paste. *Food Control* **18**: 1518-1523.

Patel R.S., Desai M.V., 1970. Control of citrus canker. *Indian Journal of Horticulture* **27**: 93-98.

Rangaswami G., Rao R.R., Lakshaman A.R., 1959. Studies on control of citrus canker with streptomycin. *Phytopathology* **49**: 224-226.

Reddy G.S., Rao P., 1960. Control of canker in citrus nurseries. *Andhra Agriculture Journal* **7**: 11-13.

Ritchie D.F., Dittspongpitch V., 1991. Copper and streptomycin resistant strains and host differentiated races of *Xanthomonas campestris* pv. *vesicatoria* in North Carolina. *Plant Disease* **75**: 733-736.

Russell A.D., Hugo W.B., 1994. Antimicrobial activity and action of silver. *Progress in Medicinal Chemistry* **31**: 351-370.

Satish S., Raveesha K.A., Janardhana G.R., 1999. Antibacterial activity of plant extract on phytopathogenic *Xanthomonas campestris* pathovars. *Letters in Applied Microbiology* **28**: 145-147.

Sawant D.M., Ghawte A.G., Jadhav J.V., Chaudhari K.G., 1985. Control of citrus canker in acid lime. *Maharashtra Journal of Horticulture* **2**: 55-58.

Shafiee A., Javidnia K., 1997. Composition of essential oil of *Zataria multiflora*. *Planta Medica* **63**: 371-372.

Sondi I., Salopek-Sondi B., 2004. Silver nanoparticles as antimicrobial agent: a case study on *Escherichia coli* as a model for Gram-negative bacteria. *Journal of Colloid Interface Science* **275**(1): 1770-1782.

Timmer L.W., 1988. Evaluation of bactericides for control of citrus canker in Argentina. *Proceedings Florida State Horticulture Society* **101**: 6-9.

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