Short Communication

INHIBITORY IMPACT OF PLANT NUTRITIONAL COMPOUNDS ON *XANTHOMONAS CITRI* subsp. *CITRI*, THE CAUSAL AGENT OF BACTERIAL CANKER OF CITRUS

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SUMMARY

Six essential plant micronutrients, copper (Cu), iron (Fe), zinc (ZN), manganese (MN), molybdenum (Mo) and boron (B) salt compounds, were investigated for their potential antibacterial activity against a group of 32 *Xanthomonas citri* subsp. *citri* (*Xcc*) isolates, as representative of the Iranian population of bacterial of citrus canker disease agents, and the sensitivity patterns of the isolates were characterized. For all tested compounds, a significant antibacterial activity against representative *Xcc* strains was determined. Bacterial growth was severely inhibited by Cu, Fe, Zn, Mn and Mo at 1/2 MIC, MIC and MBC concentrations, whereas B had only a moderate to slight impact. Among the salt compounds, Zn had the lowest MIC (0.6-1.25 mM) and MBC (2.5 mM). The highest MIC and MBC values were obtained for Mo at the dose of 30-40 mM and 40-50 mM, respectively. The results showed that 62.5% of the tested bacterial isolates were less susceptible to Fe, Zn, Mn and Mo and more susceptible to Cu and B. Nevertheless, only 37.5% of them showed a high degree of susceptibility to most of the compounds.

Key words: Xanthomonas citri subsp. *citri*, bacterial citrus canker disease, micronutrients, antibacterial activity

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Asiatic citrus canker (ACC), a serious disease caused by *Xanthomonas citri* subsp. *citri* [syn. *X. citri* pv. *citri* Gabriel *et al.* (1989)] is characterized by distinctive necrotic, erumpent lesions on the leaves, stems and fruits. Severe infections can cause a range of symptoms such as defoliation, blemished fruit, premature fruit drop, twig dieback and general tree decline (Dewdney *et al.*, 2012). ACC is now becoming a disease of high economic impact due to damages to a number of citrus species and rutaceous plants in many citrus-growing countries (Graham *et al.*, 2004). Despite of the considerable economical impact on agriculture, no effective strategy has been developed to counteract the effects of the malady. Although the application of copper-based bactericides such as copper oxychloride and Bordeaux mixture and antibiotics like streptomycin and agrimycin has already been reported against *Xcc* (Graham *et al.*, 2004), the emergence of resistant bacterial strains (Rinaldi *et al.*, 2000) and the concern about bactericide and fungicide residues has prompted the development of alternative compounds to control citrus canker. Application of organic and inorganic salts, Generally Recognized as Safe (GRAS) compounds, is an interesting alternative to control plant diseases (Talibi *et al.*, 2011). Recent reports have indicated that certain organic and inorganic salts inhibit the growth of many pathogens (Mimee *et al.*, 2011; Talibi *et al.*, 2011). Copper (Cu), iron (Fe), zinc (Zn), manganese (Mn), molybdenum (Mo) and boron (B) are essential micronutrients which play crucial roles in plant metabolism and can also increase plant tolerance to pathogens (Graham and Webb, 1991). This suggested to investigate the effectiveness against ACC of salts of Cu, Fe, Zn, Mn, Mo and B.

Thirty two strains of *Xcc* obtained from the National Institute of Genetic Engineering and Biotechnology (NIGEB, Tehran, Iran) were cultured in nutrient agar (NA) and grown at 28°C for 24 h. Single colonies were transferred to yeast extract peptone (YP) medium, grown at 28°C for 24 h to log phase, pelleted at 10,000 *g* for 20 min and resuspended in distilled water to reach to an OD_{600}

Table 1. The inhibitory effect of salt compounds on thirty two representative strains of *X. citri* subsp. *citri.* Each value in this table is presented as mean of three replicates ± standard error.

	Inhibition zone in mm											
	50 mM/disc						100 mM/disc					
Strains	Cu	Fe	Zn	Mn	Mo	B	Cu	Fe	Zn	Mn	Mo	B
NIGEB221	20.6±2.2	18.6±2.2	30.3±2.1	13.6±1.2	9.0±2.0	0.0±0	23.6±2.3	21.3±2.1	33.0±2.2	16.0±2.2	12.0±2.2	0.0±0
NIGEB220	21.3±1.3	18.6±2.2	31.0±2.5	10.0±1.3	9.0±2.2	0.0±0	23.6±2.2	21.3±1.3	34.0±2.4	12.0±2.0	10.0±2.4	0.0±0
NIGEB275	16.2±2.2	16.0±1.3	35.0±2.4	9.0±2.1	7.0±2.3	0.0±0	19.0±2.2	19.0±2.4	38.0±2.6	12.0±2.2	9.0±2.0	0.0±0
NIGEB317	20.6±2.0	18.0±2.1	40.0±3.2	10.6±1.2	11.0±1.2	0.0±0	24.3±3.1	21.0±1.2	45.0±1.2	13.0±2.0	14.0±1.2	0.0±0
NIGEB276	17.0±2.3	18.3±1.2	37.0±1.4	8.0±1.8	7.0±3.2	0.0±0	19.6±1.2	20.3±1.3	40.0±2.2	9.0±2.4	8.0±2.1	0.0±0
NIGEB031	23.0±2.1	18.3±2.3	39.6±2.2	8.0±1.4	7.3±1.2	0.0±0	25.6±2.2	22.0±2.3	42.0±1.6	10.0±1.0	9.0±1.1	0.0±0
NIGEB362	16.5±2.2	12.3±2.2	23.5±1.7	9.0±1.2	8.0±2.2	0.0±0	15.6±1.1	15.0±1.2	26.0±2.3	11.3±1.2	9.0±2.4	0.0±0
NIGEB380	21.0±1.2	17.6±1.3	35.0±2.0	11.0±2.2	13.0±2.1	0.0±0	24.0±2.2	22.0±2.0	37.0±1.4	13.0±2.4	17.0±2.1	0.0±0
NIGEB389	18.3±2.3	18.0±2.1	27.0±2.2	9.0±2.0	7.0±1.2	0.0±0	21.0±1.2	21.0±1.3	29.0±1.2	12.0±2.3	9.0±1.2	0.0±0
NIGEB375	20.6±2.3	20.6±2.2	32.2±2.3	9.0±2.3	10.0±1.2	0.0±0	23.6±2.2	23.0±2.2	34.6±3.2	12.0±1.1	12.3±1.2	0.0±0
NIGEB323	21.5±1.2	17.3±2.2	23.3±2.3	15.5±1.2	8.0±2.2	0.0±0	24±1.6	19.3±1.2	25.6±1.3	18.0±1.2	10.0±1.0	0.0±0
NIGEB392	21.0±1.2	17.5±1.0	34.0±2.2	10.3±1.1	8.0±1.2	0.0±0	23.6±2.2	20.3±2.2	35.6±2.0	14.0±1.2	10.0±1.1	0.0±0
NIGEB231	23.0±2.2	22.0±1.2	41.2±2.2	9.0±1.1	7.0±1.2	0.0±0	25.0±1.2	24.0±1.2	45.3±2.2	12.0±1.0	9.0±1.2	0.0±0
NIGEB211	23.6±2.2	16.0±1.1	38.5±2.2	13.5±1.3	8.0±1.2	0.0±0	28.6±2.2	18.0±1.2	40.6±2.2	16.6±1.2	10.0±1.1	0.0±0
NIGEB310	22.3±1.2	19.0±1.3	30.6±2.2	10.3±1.2	7.0±1.2	0.0±0	24.5±2.2	20.6±2.3	33.3±2.2	13.3±1.2	9.0±1.1	0.0±0
NIGEB385	18.0±1.2	35.0±2.2	35.0±2.2	8.0±1.1	7.0±0.9	0.0±0	20.6±1.2	37.6±1.4	37.4±1.2	10.0±1.0	8.0±1.2	0.0±0
NIGEB384	20.3±2.2	18.2±1.3	41.3±2.2	9.0±1.1	7.3±1.2	0.0±0	24.0±1.3	20.3±2.2	45.0±2.2	11.0±1.2	8.0±1.2	0.0±0
NIGEB383	16.3±0.9	16.2±1.2	40.3±2.2	14.0±1.4	8.0±1.2	0.0±0	18.2±1.3	18.0±1.2	44.3±2.2	16.6±1.2	10.0±1.1	0.0±0
NIGEB306	21.3±2.2	18.0±1.3	40.3±2.2	10.0±1.2	8.0±0.8	0.0±0	23.5±2.2	20.0±1.2	44.6±2.2	12.0±1.3	10.0±1.2	0.0±0
NIGEB381	20.3±2.3	18.3±1.2	30.5±2.2	8.0±1.0	7.0±1.1	0.0±0	23.2±2.2	21.0±2.2	33.6±2.2	10.0±1.3	9.0±2.3	0.0±0
NIGEB387	21.0±2.2	18.0±1.4	32.0±2.3	9.0±1.3	7.0±1.2	0.0±0	23.3±2.3	20.0±1.3	36.0±2.3	12.0±1.4	9.0±1.1	0.0±0
NIGEB247	21.0±1.2	18.0±1.2	35.0±2.3	8.0±1.1	7.0±1.1	0.0±0	23.3±2.3	20.6±2.2	37.6±2.3	10.0±1.6	8.0±1.1	0.0±0
NIGEB281	19.3±1.1	16.3±1.3	35.3±2.2	10.6±1.3	8.0±1.4	0.0±0	21.3±1.8	18.2±1.1	38.6±2.3	14.3±1.5	10.0±1.3	0.0±0
NIGEB228	21.0±1.7	18.0±1.3	35.0±2.3	8.0±1.1	6.0±1.1	0.0±0	23.3±2.2	20.6±1.7	37.5±2.3	10.0±1.2	8.0±1.5	0.0±0
NIGEB277	21.0±2.4	18.0±1.6	35.0±2.3	8.0±1.1	6.0±1.0	0.0±0	23.2±1.8	20.6±2.2	37.0±2.5	10.0±1.1	8.0±1.3	0.0±0
NIGEB254	21.0±1.4	19.0±1.9	30.0±2.3	8.0±1.3	7.0±1.3	0.0±0	23.6±2.2	21.0±1.7	31.0±2.2	10.6±1.3	9.0±1.6	0.0±0
NIGEB386	21.0±2.1	19.0±1.4	30.0±2.2	8.0±1.1	7.0±1.2	0.0±0	23.6±2.4	21.0±1.3	31.0±1.8	10.0±1.6	9.0±1.1	0.0±0
NIGEB048	21.3±2.6	19.6±1.3	31.0±3.3	8.0±1.3	7.0±1.1	0.0±0	23.5±2.4	21.3±1.2	33.6±2.3	11.0±0.9	9.0±1.3	0.0±0
NIGEB170	22.6±2.3	16.6±1.4	24.3±2.2	8.0±1.1	8.0±1.0	0.0±0	24.3±1.5	19.0±1.2	26.0±1.1	11.0±0.8	9.6±0.7	0.0±0
NIGEB273	21.0±2.1	18.0±1.1	35.0±2.3	8.5±0.7	7.2±1.9	0.0±0	23.0±2.2	20.6±1.4	37.0±2.1	10.0±1.2	8.0±1.4	0.0±0
NIGEB088	22.0±2.0	17.0±1.2	38.0±2.3	9.0±1.8	8.0±1.1	0.0±0	24.0±1.4	19.0±1.1	40.0±2.2	16.0±1.4	10.0±1.0	0.0±0
LMG9322	22.0±1.9	17.0±1.2	38.6±2.2	9.0±0.3	8.0±1.5	0.0±0	24.0±2.4	19.0±0.7	40.0±2.2	16.0±1.1	10.0±0.9	0.0±0

of 0.4 equivalent to 10^6-10^8 CFU ml^{-1}. Copper (II) sulfate ($CuSO_4$), ferrous sulfate ($FeSO_4 \cdot 7H_2O$), zinc sulfate ($ZnSO_4 \cdot 7H_2O$), manganese (II) sulfate ($MnSO_4 \cdot H_2O$), molybdic acid ($MoO_3 \cdot H_2O$) and boric acid (H_3BO_3) (Merck Millipore, Germany) were used as inorganic source of plant microelements. Stock solutions of each reagent were prepared at a concentration of 500 mM and sterilized by filtering through a sterile 0.20 µm Minisart filter. Salt compounds were screened *in vitro* for their antibacterial activity against the 32 *Xcc* isolates using the disc diffusion method (Bauer *et al*., 1966). To this aim 100 µl of each bacterial suspension was spread evenly over NA plates with sterile glass rods and allowed to dry. Analytical blank paper disks 6.4 mm in diameter (PATAN TEB, Iran)

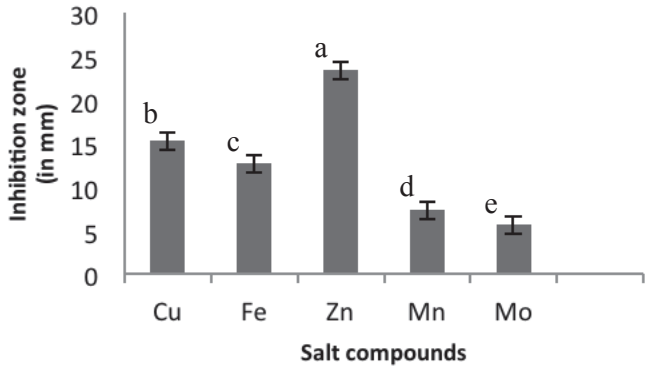

Fig. 1. The comparison between inhibition zone caused by Cu, Fe, Zn, Mn, Mo and B salts against thirty two strains of *X. citri* subsp. *citri.* Values with different letters are significantly different according to Duncan (p = 0.05) test. The inhibition zones caused by Zn were mostly larger than those of other compound examined and there was not any inhibition zone about B element. Bars indicate ± standard error.

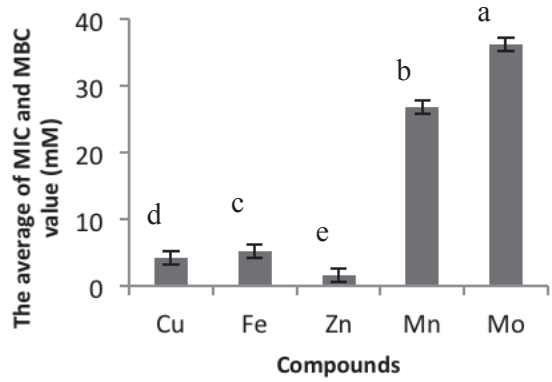

Fig. 2. The average MICs and MBCs values of Cu, Fe, Zn, Mn and Mo compounds on thirty two strains of *X. citri* subsp. *citri.* Values with different letters are significantly different according to Duncan (p = 0.05) test. Zn and Mo compounds had the lowest and highest MIC and MBC values respectively. Bars indicate ± standard error.

saturated with 50 µl of each compound at the concentration of 50 and 100 mM, or distilled water as control, were placed on the surface of the medium and tapped likely to ensure even contact. All plates were incubated at 28°C for 24 h. Each experiment was carried out in triplicate, and the antibacterial activity was determined by measuring the diameter of the inhibition zone in mm. The minimum inhibitory concentration (MIC) of the salt treatments was determined by serial dilutions (Jawetz *et al*., 1980) from a concentration of 0.1 to 200 mM in a total volume of 200 µl against *Xcc* strains. Bacterial suspensions (100 µl) containing 10^6-10^8 CFU ml^{-1} were placed into a sterile 96-well plate (GRE 961), incubated at 28°C and observed for bacterial growth for 24 h. After incubation, samples with no visible bacterial growth compared to the control (bacterial suspension with no treatment) were taken to represent the MIC value of the given salt treatments in mM. To confirm the experiment, 5 ml resazurin (7-Hydroxy-3H-phenoxazin-3-one 10-oxide) solution, prepared by dissolving 4 mg resazurin sodium salt powder in 10 ml distilled water, was added to each well and color change was assessed visually. The lowest concentration of the salt tested at which color change had occurred was taken as the MIC value (Sarker *et al*., 2007). For determining the minimum bactericidal concentration (MBC), NA plates were divided into eight sections. One hundred µl of the bacterial suspension from wells were recultured on each section. The plates were incubated at 28°C for 48 h and MBC was determined at the lowest concentration where no bacterial growth was observed (Jawetz *et al*., 1980). The experiment was designed to monitor the growth of bacterial strains in the presence of salt treatments using microtiter broth method (du Toit and Rautenbach, 2000). Sterile 96-well plates (GRE 961) were labeled according to bacterial strain and compound type and concentration. One hundred µl of each bacterial suspension in the mid-exponential phase of growth were treated with different concentration of salt treatments in a total volume of 200 µl YP medium and one well of the plate in each row was filled with 100 µl of non treated bacterial suspension as control. Plates were sealed, placed on a rotary shaker set at 180 rpm and 28°C and the optical density was measured (OD_{600} nm) by a micro reader (Infinite 200 Nano Quant, Tecan, Switzerland) after incubation at 0, 2, 4, 8, 16 and 24 h. The percentage of growth ratio (GR%) of strains at 24 h after incubation was calculated by the following formula:

$$GR\% = (OD_{treatment}/OD_{blank}) \times 100.$$

Bacterial strains in response to inhibitory effects of salt treatments (GR%) were classified using Gene cluster software 3.0 versions and Tree View version 1.60 according to the software instruction manual (Amini *et al*., 2012). For this analysis, similarity matrix of Spearman Rank Correlation and Average Linkage Clustering were used. The Statistic Analysis System program (SAS/IML, version 9.1) was used for analysis of variance (ANOVA) followed by Duncan test at 0.05 for mean separation.

Analysis of the antibacterial activity of selected concentrations of salt treatments showed that all treatments, except for B, had a direct antibacterial activity against all 32 tested *Xcc* strains at the concentrations of 50 and 100 mM (Table 1). However, there was a significant difference in the size the inhibition zone between compounds (Fig. 1). In fact, inhibition diameters induced by the 50 mM concentration were 16.2-23.6 (Cu), 12.3-22.6 (Fe), 23.3-41.3 (Zn), 8-15.5 (Mn) and 7-13 (Mo) mm, respectively, showing that of the six tested compounds, Zn was the most effective. Table 1 shows also that, a higher concentration (100 mM), the six compounds elicited a more important inhibitory effect. It is noteworthy that B had no inhibitory effect even at the highest concentration tested (500 mM).

Reduction in disease severity has been reported upon application of Cu, Mn and B salts against powdery mildew in cucumber (Reuveni *et al*., 1998), gray mould on

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of salt compounds against thirty two strains of *X. citri* subsp. *citri.*

	MIC (mM)						MBC (mM)					
Strains	Cu	Fe	Zn	Mn	Mo	B	Cu	Fe	Zn	Mn	Mo	B
NIGEB221	2.5	5	0.6	20	30	0	5	10	2.5	30	40	0
NIGEB220	2.5	5	0.6	20	30	0	5	10	2.5	30	40	0
NIGEB275	2.5	10	0.6	20	30	0	5	20	2.5	30	40	0
NIGEB317	2.5	10	0.6	20	30	0	5	20	2.5	30	40	0
NIGEB276	2.5	10	0.6	20	40	0	5	20	2.5	30	50	0
NIGEB031	5	5	0.6	20	30	0	5	10	2.5	30	40	0
NIGEB362	2.5	5	0.6	20	30	0	5	10	2.5	30	40	0
NIGEB380	2.5	5	0.6	20	30	0	5	10	2.5	30	40	0
NIGEB389	5	10	0.6	20	30	0	10	20	2.5	30	40	0
NIGEB375	5	10	0.6	20	30	0	10	20	2.5	30	40	0
NIGEB323	5	5	0.6	20	30	0	10	10	2.5	30	40	0
NIGEB392	5	5	0.6	20	30	0	10	10	2.5	30	40	0
NIGEB231	2.5	5	0.6	20	30	0	5	10	2.5	30	40	0
NIGEB211	2.5	5	0.6	30	30	0	5	10	2.5	40	40	0
NIGEB310	2.5	5	0.6	20	30	0	5	10	2.5	30	40	0
NIGEB385	2.5	5	0.6	20	30	0	5	10	2.5	30	40	0
NIGEB384	2.5	5	0.6	20	30	0	5	10	2.5	30	40	0
NIGEB383	2.5	10	0.6	20	30	0	5	20	2.5	30	40	0
NIGEB306	2.5	5	0.6	20	40	0	5	10	2.5	30	50	0
NIGEB381	2.5	5	0.6	20	30	0	5	10	2.5	30	40	0
NIGEB387	2.5	5	0.6	20	30	0	5	10	2.5	30	40	0
NIGEB247	5	5	1.25	20	30	0	10	10	2.5	30	40	0
NIGEB281	5	5	1.25	20	30	0	10	10	2.5	30	40	0
NIGEB228	5	5	1.25	20	30	0	10	10	2.5	30	40	0
NIGEB277	2.5	5	0.6	20	30	0	5	10	2.5	30	40	0
NIGEB254	2.5	5	1.25	20	30	0	5	10	2.5	30	40	0
NIGEB386	2.5	5	0.6	20	30	0	5	10	2.5	30	40	0
NIGEB048	5	10	0.6	20	30	0	10	20	2.5	30	40	0
NIGEB170	5	5	0.6	20	30	0	10	10	2.5	30	40	0
NIGEB273	5	5	0.6	30	30	0	10	10	2.5	40	40	0
NIGEB088	2.5	5	0.6	20	30	0	5	10	2.5	30	40	0
LMG9322	2.5	10	0.6	20	30	0	5	20	2.5	30	40	0

table grapes caused by *Botrytis cinerea* (Qin *et al*., 2010), *Plasmodiophora brassicae* in crucifers and *Fusarium solani* in bean (Graham and Webb, 1991) and *Blumeria graminis* in wheat (Marschner, 1995). Zinc was also found to have a toxic effect on the pathogen directly (Graham and Webb, 1991). Application of Zn was reported to reduce *Fusarium graminearum* infections and root rot caused by *Gaeumanomyces graminis* in wheat (Graham *et al*., 1991; Grewal *et al*., 1996). The sensitivity of Mexican strains of the causal agent of bacterial spot of pepper (*Xanthomonas. campestris* pv. *vesicatoria*) to Zn has also been reported (Adaskaveg and Hine, 1985), whereas conidial germination and development of *Helminthosporium solani* was totally inhibited by Fe salts (Mimee *et al*., 2011). Fe could also control or reduce the severity of several diseases such as rust and smut in wheat and *Colletotrichum musae* in banana (Graham, 1983; Graham and Webb, 1991). Boron was effective in controlling gray mold on table grapes (Qin *et al*., 2010), which contracts with the total lack of inhibitory activity shown in the present investigation.

As to the MIC and MBC values (Table 2), Zn showed the lowest MIC (0.6-1.25 mM) and MBC (2.5 mM), whereas the highest values were afforded by Mo at the dose of 30-40 mM and 40-50 mM, respectively (Fig. 2). The differences between the mean of MICs calculated for Cu, Fe, Zn, Mn and Mo were found to be statistically significant

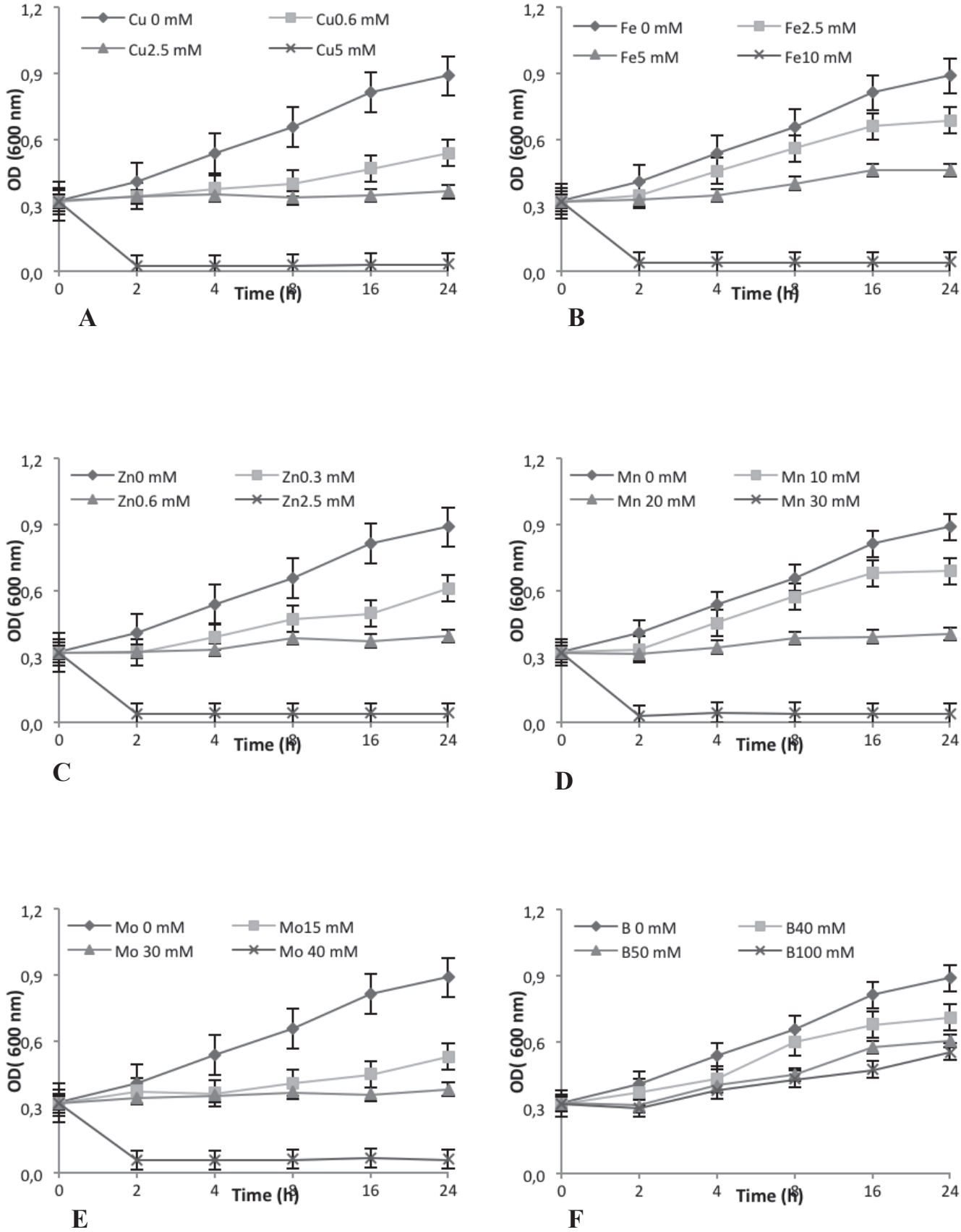

Fig. 3. Representative growth curves (change in OD_{600} over time) for *X. citri* subsp. *citri* strain NIGEB088 exposed to Cu (A), Fe (B), Zn (C), Mn (D), Mo (E) and B (F) concentrations of 0 (bacterium), 0.1, 0.3, 0.6, 2.5, 5, 10, 20, 30 and 50 mM at 0, 2, 4, 8, 16 and 24 hours after incubation. The MIC value shown on the graphs for Cu, Fe, Zn, Mn and Mo is 2.5, 2.5, 0.6, 20 and 30 mM and MBC values are 5, 10, 2.5, 30 and 40 mM respectively. Boron compounds did not inhibit the growth completely. The same approach was used to determine the MIC for other strains. Bars indicate ± standard error.

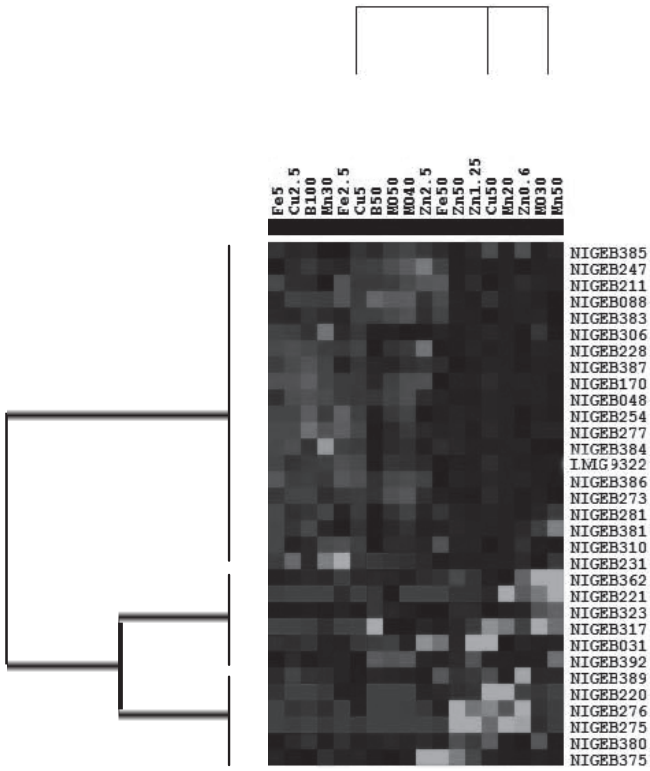

Fig. 4. The cluster analysis of thirty two strains of *X. citri* subsp. *citri* using GR% at the range of MIC and MBC values. The strains were comprised in two distinguished groups based on analyzing by gene cluster software. Group 1 contained 62.5 % of all strains (NIGEB 385, 247, 211, 088, 383, 306, 228, 387, 170, 048, 254, 277, 384, 386, 273, 281, 310, 231 and LMG 9322) and group 2 was consisted of 37.5% of all strains contained two sub groups (NIGEB 362, 221, 323, 317, 031, 392 and NIGEB 389, 220, 276, 275, 380, 375).

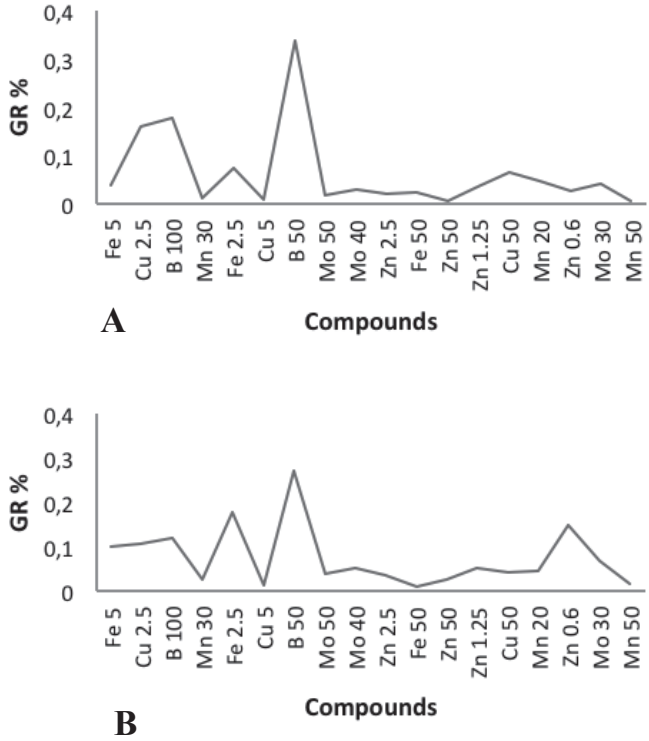

Fig. 5. Growth ratio patterns of thirty two strains of *X. citri* subsp. *citri* grouped in two clusters. The patterns of strains in group 1 (A) were different from group 2 (B). The strains in group 1 (with a higher GR %) were less susceptible to Fe, Zn, Mn and Mo but more susceptible to Cu and boron and strains in group 2 showed a high level of susceptibility to most of the compounds.

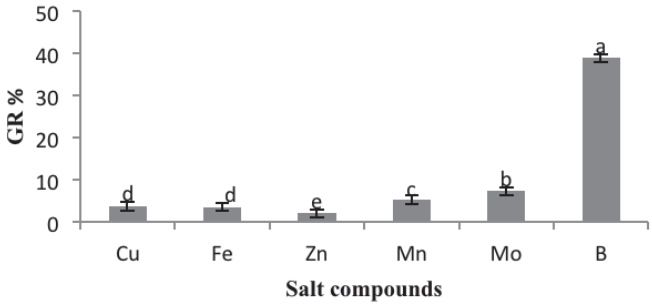

Fig. 6. The average of the bacterial growth percentage calculated for thirty two strains of *X. citri* subsp. *citri* at the presence of Cu, Fe, Zn, Mn, Mo and B at concentration of 50 mM. Values with different letters are significantly different according to Duncan ($p = 0.05$) test. Among the tested compounds Zn and B had the highest and the lowest inhibitory activity respectively. Bars indicate ± standard error.

($P < 0.05$). Boron did not completely inhibit strain growth at 50 and 100 mM, thus it did not have any bactericidal effect.

The growth of bacterial strains was determined by monitoring the optical density of cultures at 600 nm in a 96-well plate after 0, 2, 4, 8, 16 and 24 h incubation (Fig. 3), then calculating the GR% of all strains in the presence of salt compounds. Except for boron, the other five salts compounds had a severely inhibitory effect, with the same pattern at concentrations of 1/2MIC, MIC and MBC. In the same conditions, boron exerted only a moderately to slightly impact on the growth of the bacterial strains. Cluster analysis of strains using GR% showed that the strains clustered in two separate groups (Fig. 4). The growth ratio patterns of strains in the groups are shown in Fig. 5, which shows that group 1 contains 62.5% of the strains while only 37.5% of them is comprised in group 2. It ensues that group strains 1 (with a higher GR%) are less susceptible to Fe, Zn, Mn and Mo but more susceptible to Cu and B. By contrast, strains of group 2 showed a high level of susceptibility to most of the compounds. The average GR% for the 32 strains in the presence of 50 mM of each experimental treatment is presented in Fig. 6.

The underlying reasons for differential responses of microorganisms to antimicrobial compounds are poorly understood, but the chemical composition of the outer cellular layers is likely to be a factor of primary importance (Russell, 2003). The other possible contributory factors may be differences in stress responses, the presence of efflux pumps and cells occurring within biofilms (Russell, 2003), so possible physiological or molecular differences

between strains may result in various behaviors of the bacterial cell membrane in response to inhibitory compounds. For all strains, an increase in concentration of salts was associated with bacterial growth inhibition in comparison to controls. The present study showed that in addition to Cu, other plant trace elements such as Fe, Zn, Mn, Mo, B and zinc in particular (with the highest diameter of inhibition, the highest bacterial growth inhibitory effect and the lowest MIC and MBC values) strongly influenced growth of *Xcc* strains and that the observed toxicity of the micronutrients resulted from direct toxic effect on the pathogen.

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