Vol. 16(6), pp. 138-147, July 2022
DOI: 10.5897/AJPS2022.2245
Article Number: EE0227569451
ISSN 1996-0824

http://www.academicjournals.org/AJPS

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African Journal of Plant Science

Full Length Research Paper

Antibacterial activity of some substances against citrus bacterial canker caused by *Xanthomonas citri* pv*. citri* (Hasse) in Burkina Faso

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Received 24 March, 2022; Accepted 8 June, 2022

Citrus bacterial canker (CBC) threatens citrus fruit production in Burkina Faso due to the lack of effective control methods. In this study, the antimicrobial effect of copper hydroxide and sulphate, the biopesticide from *Bacillus amyloliquefaciens*, the essential oil of *Ocimum gratissimum* and aqueous extracts of *Eucalyptus camadulensis, Azadirachta indica* and *Cymbopogon citratus* were tested *in vitro* and *in vivo* against *Xanthomonas cirri* pv*. citri* (Hasse) (*Xcc*890) strain from Burkina Faso. The dilution method in solid medium and macro-method dilution in liquid medium were, respectively used in order to determine the minimum bactericidal concentration (MBC) and the minimum inhibitory concentration (MIC). Hydroxide and sulphate copper tested at 25 mg/ml induced 13 mm in diameter of inhibition zone, followed by *B. amyloliquefaciens* at 25 µl/ml (11.6±3.9 mm). *O. gratissimum* induced 10.5 ± 0.11 mm of inhibition zone at 5 µl/ml. Aqueous extract of *E. camadulensis* at 100 mg/ml induced 11.4 ± 1 mm of inhibition diameter. Based on MBC/MIC ratio, copper hydroxide and sulphate, *O. gratissimum* and *E. camadulensis* have a bactericidal effect *in vitro. In vivo* test, copper products at 25 mg/ml and *O. gratissimum* at 10 µl/ml were the most effective and significantly reduced the CBC incidence. These substances can be evaluated under natural conditions in order to determine their effectiveness against CBC.

Key words: *Citrus* species, citrus bacterial canker, *Xanthomonas citri* pv*. citri,* antibacterial activity, substances.

INTRODUCTION

Citrus bacterial canker (CBC) caused by *Xanthomonas citri* pv. *citri* (*Xcc*) represents the most devastating bacterial disease on citrus production through the world (Salifou and Bounou, 2020). The disease causes defoliation, fruit cankers, a premature fall of fruit and a general withering of trees (Gottwald et al., 2002; CABI,

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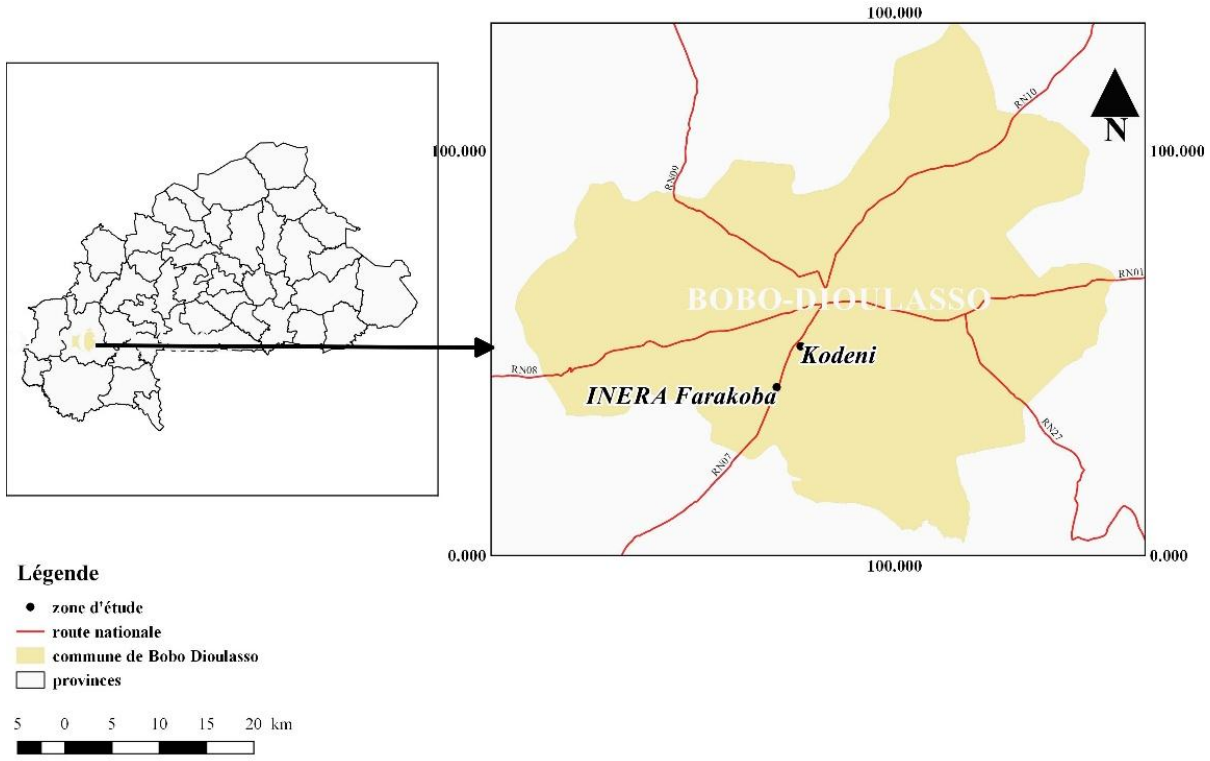

Figure 1. Location of *Xcc* 890 strain isolation site.
Source: Authors

2019). There are no highly efficient strategies for controlling CBC disease. In Burkina Faso, CBC was reported on all commercial citrus cultivars in the provinces of Comoé, Houet and Kénédougou (Juhasz et al., 2013). In endemic areas, the quarantine and eradication are the major means of control against *Xcc* (OEPP/CABI, 1990). However, the use of windbreaks, destruction of infected branches and spraying of copper-based products during periods of susceptibility, can reduce the severity of the disease (Civerolo, 1984; Behlau, 2021).

Indeed, copper-based products, despite their effectiveness (Stall et al., 1980; Idrissou-Touré et al., 2017), have an adverse effect on human and environmental health (Villeneuve, 2008; Damien et al., 2017). In addition, their repetitive and uncontrolled use causes resistance phenomenon in pathogens.

In order to prevent major damage caused by CBC, alternative less harmful tools have been extensively studied in recent years (Oliveira et al., 2021; Raveau, 2020). For example, several studies have shown that many plants from the West African flora have an enormous biocidal potential (toxic, repellent, anti-appetizing) against a broad spectrum of pests. They can be used in the form of aqueous extracts or essential oils (Saha et al., 2013; Zombré et al., 2015). In Burkina Faso, despite the high prevalence of CBC and the resulting crop losses, no means of control, including the use of copper-based products, have been deployed. Wonni et al. (2016) showed that aqueous extract and essential oil of *Eucalyptus camaldulensis* have an antibacterial activity *in vitro* test against both pathovars of *Xanthomonas oryzae*. The results of Rabbee et al. (2021) showed that ethyl acetate extract from *Bacillus velezensis*, have antibacterial activity against *Xcc* wildtype strains, in contrast to *Bacillus amyloliquefaciens* strains which had no antibacterial activity.

In order to promote an effective control of *Xcc*, the present study aimed to evaluate *in vitro* and *in vivo,* the antibacterial activity of some chemical, biological substances, plant aqueous extracts, and essential oils.

MATERIALS AND METHODS

Bacterial strain and inoculum's preparation

Strain *Xcc* 890 was isolated in 2020 from samples of tangelo species (*Citrus reticulata* × *Citrus paradisi*) from the citrus orchard of the locality of Kodeni which is located in the commune of Bobo-Dioulasso (Figure 1), on the Bobo-Banfora axis (National Road No. 7). The geographic coordinates of the site are 11°7'40.5372" North Latitude, 4°19'6.34332" West Longitude. The inoculum was prepared from a 24 h bacterial culture. It was suspended in 5 ml of 0.85% physiological NaCl solution and adjusted to 10^8 bacterium/ml using a spectrophotometer at A_{600nm}. The different activities were

Table 1. Spectrum of substances concentration used in this study.

Substance	Concentration					
	C1	C2	C3	C4	C5	C6
Copper hydroxide (mg/ml)	25	12.5	6.25	3.12	1.56	0.78
Copper sulfate (mg/ml)	25	12.5	6.25	3.12	1.56	0.78
Essentiel oil of *Ocimum gratissimum (*µl/ml)	5	2.5	1.25	0.62	0.31	0.15
Aqueous extracts of *Azadirachta indica* (µl/ml)	100	50	25	12.5	6.25	3.12
Aqueous extracts of *Eucalyptus camadulensis* (µl/ml)	100	50	25	12.5	6.25	3.12
Aqueous extracts of *Cymbopogon citratus* (µl/ml)	100	50	25	12.5	6.25	3.12
Bacillus amyloliquefaciens (Serenade) (µl/ml)	25	12.5	6.25	3.12	1.5	0.78
Thymol gamma terpène (Neco 50EC) (µl/ml)	50	25	12.5	6.25	3.12	1.56
Thymol eugenol (Proraly 50EC) (µl/ml)	50	25	12.5	6.25	3.12	1.56

Source: Authors

carried out in the bacteriology laboratory of INERA/FARAKOBA.

Substances tested

Five types of substances were tested. They were (i) Chemicals; sulphate and hydroxide copper, (ii) plant aqueous extracts; of *Azadirachta indica* (A.) JUSS, *Cymbopogon citratus* (D.C.) STAFF, and *E. camadulensis* DEHNH, (iii) essential oils; *Ocimum gratissimum* LINNE, (iv) biological substance derived from *B. amyloliquefaciens* supplied by BAYER company and (v) formulations based on essential oils that were Neco 50EC and Proraly 50EC from Côte d'Ivoire.

Preparation of aqueous plant extracts

The aqueous extracts of leaves of *A. indica* (A.) JUSS., *C. citratus* (D.C.) STAFF and *E. camadulensis* DEHNH, were prepared according to the method described by Guédé-Guina et al. (1996).

The leaves were collected at the Farako-Ba station with coordinates Longitude 4°20' West, Latitude 11°6' North and an altitude of 405 m. The leaves were firstly dried in the shade for two weeks, and then powdered using a mortar. The powders were macerated in the proportion of 1 g of powder/1 ml of distilled water and the suspension was kept for 24 h. The mixture was then, centrifuged at 4000 rpm for 10 min and the supernatant was sterilized in order to remove contaminants. The final substrate was stored in the refrigerator for its use.

Concentration range

Six concentrations per substance were used in this study. They were prepared by the method of double dilution in according to a geometric progression of ½ reason (Toty et al., 2013) from a maximal concentration (C1) to a minimal concentration (C6) (Table 1).

Efficiency test *in vitro*

It was performed using the standard disc or antibiogram method (Abo-Elyousr and Asran, 2009). Bacterial suspension of 40 µl at 10^8 CFU/ml was spread uniformly over the entire surface of the LPGA solid medium (LPGA for 1 L: Yeast 7 g, Peptone 7 g, Glucose 7 g, Agar 18 g) contained in Petri dishes. After drying, sterile blotting paper discs of 6 mm diameters were placed and inoculated with 10 µl of each substance. A negative control was inoculated with sterile distilled water. Petri dishes were firstly incubated for 1 h at room temperature, and then at 30°C in inverted position into incubator. After 72 h incubation, the diameters of inhibition (ID) zones were measured.

The efficacy of each substance was determined based on the criteria of Ponce et al. (2003) as follows: (i) ineffective: ID <8 mm, (ii) effective: 9 mm < ID <14 mm; (iii) very effective: 15 mm <ID <19 mm, and (iv) highly effective: ID > 20 mm.

Determination of antibacterial parameters

It was carried out using the technique of microdilution in nutrient liquid. This technique consisted of inoculate in a standardized inoculum, and decrease concentrations of the tested substances. For this, a series of seven test tubes, each containing 3 ml of LPGA nutrient liquid, were inoculated with 50 µl of bacteria inoculum at 10^8 colonies/ml, and then 50 µl of each substance according to the concentrations defined in Table 1 except for the control tube. The tubes were placed at 28°C under shaking for 24 h, and then at 28°C for 72 h in a bacteriological incubator. Three parameters were assessed in the following.

Bacterial growth (T_{survey})

It was determined to measure the optical density (DO) of each tube using spectrophotometer before and after incubation and to calculate with the formula used by Moroh et al. (2008).

T_{survey} (x) = (T_i -T_f) / (T_{it} –T_{ft}),

where T_i: initial DO; T_f: final DO; T_{it}: initial DO of control without substance; T_{ft}: final DO of control without substance.

Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

They were determined to assess the bactericidal or bacteriostatic effect (Berche et al., 1991) of each substance by calculating the MBC/MIC ratio as follows:

(1) bactericidal if MBC/MIC ≤ 2;
(2) bacteriostatic if MBC/MIC > 2

Table 2. Inhibition's diameter (mm) of the bacterial growth induced by the tested substances.

Concentration	Inhibition diameter (mm)								
	Copper hydroxide	**Copper sulphate**	**Serenade**	***Ocimum. gratissimum***	**Neco 50EC**	**Proraly 50EC**	***Azadiractha indica***	***Cymbopogon citratus***	***Eucalyptus camadulensis***
C1	13.3±1.6^{a}	13±01.3^{a}	11.6±3.9^{a}	10.5±0.11^{a}	9.4±1.3^{a}	9.6±0.9^{a}	10.1±1.3^{a}	10.9±1.2^{a}	11.4±1^{a}
C2	12±4.5^{a}	10.7±1.2^{b}	10.4±2.5^{ab}	8.9±0.07^{b}	6±0.0^{b}	8.7±0.9^{b}	7.6±1.1^{b}	8±0.7^{b}	8.9±0.9^{b}
C3	9.1±2.9^{b}	9.2±1.0^{c}	8.7±1.1^{c}	8±0.11^{c}	6±0.0^{b}	7.7±0.7^{c}	7.3±0.9^{b}	7.5±0.0^{C}	7.9±1.1^{b}
C4	7.2±1.1^{c}	8.6±0.7^{c}	7.1±0.9^{d}	6±0.00^{d}	6±0.0^{b}	6±0.0^{d}	6±0.0^{e}	6±0.0^{d}	6.4±1.0^{c}
C5	6.4±0.7^{C}	7.6±0.9^{d}	6±0.0^{e}	6±0.00^{d}	6±0.0^{b}	6±0.0^{d}	6±0.0^{e}	6±0.0^{d}	6±0.0^{d}
C6	6.1±0.3^{c}	7±0.8^{d}	6±0.0^{e}	6±0.00^{d}	6±0.0^{b}	6±0.0^{d}	6±0.0^{e}	6±0.0^{d}	6±0.0^{d}
P-value	0.001	0.002	0.001	0.003	0.005	0.005	0.003	0.003	0.001

a-e: Different letters indicate statistically relevant differences among inhibition diameters within each treatment using the Tukey test ($p < 0.05$).
Source : Authors

***In vivo* test**

Young plants of the tangelo species with leaves infected by CBC, from nurseries in the city of Orodara, were used under greenhouse conditions, to test the effective concentrations of the substances which were found to be effective *in vitro*. Therefore, copper hydroxide and sulfate (25 and 12.5 mg/ml), HE of *O. gratissimum* (5 and 10 µl/ml), the aqueous extract of *E. camadulensis* (50 and 100 mg/ml), and *B. amyloliquefaciens* formulation (30 and 60 µl/ml) were tested every 15 days.

Before foliar treatment with the substances, we counted (i) the number of symptomatic leaves per plant, (ii) the proportion of canker on each symptomatic leaf per plant, and (iii) the number of symptomatic leaves of the plant. These data were used to calculate the foliar incidence and severity of CBC according to the following formulae defined respectively by Idroussou-Touré et al. (2017) and Mayee and Datar (1986).

Disease incidence (%) = (NSL/NTL) × 100

where NSL: the number of symptomatic leaves; NTL: the number of total leaves.

The severity was evaluated using the scale of Lakshmi et al. (2011).
Severity = Addition of numerical notation × 100 / Number of examined unit × Maximal note.

Statistical analysis

The analysis of variance and Tukey's test were carried out with the Minitab18 software in order to compare the effectiveness of the substances used at different doses *in vitro* and *in vivo*, but also to compare the variations between MIC and BMC.

RESULTS

Sensitivity of the *Xcc* strain to the tested substances

All the tested substances showed an inhibitory effect at the different concentrations used, with a very remarkable effect of copper hydroxide at 12.5 mg/ml, inducing 13.3 ± 1.6 mm of inhibition diameter (Table 2). In general, the inhibition diameter was proportional to the concentration of each tested substance. In fact, the highest inhibition diameters were obtained with the maximum concentrations (C1).

Efficiency of the different tested concentrations

Tables 3 to 6 show the efficiency of the antibacterial activity of each substance in terms of the tested concentrations. The products based on copper were efficient from the concentration of 3.12 mg/ml for copper hydroxide and 6.25 mg/ml for copper sulphate (Table 3). The antibacterial activities of Proraly 50EC and Neco 50EC were efficient, respectively from 25 and 50 µl/ml concentrations (Table 4). Below these doses, they became inefficient. Furthermore, the antibacterial activity of the formulation based on *B. amyloliquefaciens* was efficient to the concentration of 6.25 µl/ml, whereas the essential oil of *O. gratissimum* was efficient from 1.5 µl/ml (Table 5). The efficiency of plant aqueous extracts was very variable according to the vegetal species. So, the aqueous extracts of *C. citratus* and *E. camadulensis* were efficient to the

Table 3. Efficiency of copper hydroxide and copper sulphate against *Xcc*.

Concentration (mg/ml)	Substance	Inhibition diameter (mm)	Efficiency
25	Copper hydroxide	13.3±1.6^{a}	Efficient
	Copper sulphate	13±1.3^{a}	Efficient
12.5	Copper hydroxide	12±4.5^{ab}	Efficient
	Copper sulphate	10.7±1.2^{bc}	Efficient
6.25	Copper hydroxide	9.1±2.9^{cd}	Efficient
	Copper sulphate	9.2±1.0^{cd}	Efficient
3.12	Copper hydroxide	8.6±0.7^{cde}	Efficient
	Copper sulphate	7.2±1.1^{def}	Inefficient
1.56	Copper hydroxide	6.6±0.8^{ef}	Inefficient
	Copper sulphate	7.6±0.9^{def}	Inefficient
0.78	Copper hydroxide	6.1±0.3^{f}	Inefficient
	Copper sulphate	7.6±0.9^{def}	Inefficient

a-f: Different letters indicate statistically relevant differences among inhibition diameters within each treatment using the Tukey test ($p < 0.05$).
Source : Authors

Table 4. Efficiency of Neco 50EC and Proraly 50EC against *Xcc*.

Concentration (µl/ml)	Substance	Inhibition diameter (mm)	Efficiency
50	Proraly 50EC	9.6±0.9^{a}	Efficient
	Neco 50EC	9.4±1.3^{ab}	Efficient
25	Proraly 50EC	8.7±0.9^{b}	Efficient
	Neco 50EC	6±0.0^{d}	Inefficient
12.5	Proraly 50EC	7.7±0.7^{c}	Inefficient
	Neco 50EC	6±0.0^{d}	Inefficient
6.25	Proraly 50EC	6±0.0^{d}	Inefficient
	Neco 50EC	6±0.0^{d}	Inefficient
3.12	Proraly 50EC	6±0.0^{d}	Inefficient
	Neco 50EC	6±0.0^{d}	Inefficient
1.56	Proraly 50EC	6±0.0^{d}	Inefficient
	Neco 50EC	6±0.0^{d}	Inefficient

a-d: Different letters indicate statistically relevant differences among inhibition diameters within each treatment using the Tukey test ($p < 0.05$).
Source: Authors

concentration of 50 mg/ml, whereas that of *A. indica* was efficient from 100 mg/ml (Table 6).

Survival rate of *Xcc*

The survival rates of *Xcc* at the different tested concentrations were inversely proportional to the increase of the concentration of each substance (Table 7). Indeed, the lowest rate of survival (-9.2±3.26%) was registered with *O. gratissimum* at 5 µl/ml. However, the highest rate of survival (124.32±31.54%) was registered with the aqueous extract of *A. indica* at 6.25 mg/ml.

Characteristics of the tested substances

The different values of Minimal Inhibitory Concentrations

Table 5. Efficiency of *Bacillus amyloliquefaciens* formulation and essential oil of *Ocimum gratissimum* against *Xcc*.

Substance	Concentration (µl/ml)	Diameter (mm)	Efficiency
Bacillus amyloliquefaciens	25	11.6±3.9[a]	Efficient
	12.5	10.4±2.5[ab]	Efficient
	6.25	8.7±1.1[c]	Efficient
	3.12	7.1±0.9[d]	Inefficient
	1.56	6±0.0[e]	Inefficient
	0.78	6±0.0[e]	Inefficient
Ocimum gratissimum	5	10.5±0.11[a]	Efficient
	2.5	8.9±0.07[b]	Efficient
	1.5	8±0.11[c]	Efficient
	0.62	0.6±0.00[d]	Inefficient
	0.31	0.6±0.00[d]	Inefficient
	0.15	0.6±0.00[d]	Inefficient

a-e: Different letters indicate statistically relevant differences among inhibition diameters within each treatment using the Tukey test ($p < 0.05$).
Source: Authors

Table 6. Efficiency of plant aqueous extracts against *Xcc*.

Concentration (mg/ml)	Plant aqueous extract	Inhibition diameter (mm)	Efficiency
100	*Eucalyptus camadulensis*	11.4±1.5[a]	Efficient
	Cymbopogon citratus	10.9±0.7[ab]	Efficient
	Azadirachta indica	10.1±0.7[bc]	Efficient
50	*Eucalyptus camadulensis*	8.9±0.9[b]	Efficient
	Cymbopogon citratus	8±0.9[b]	Efficient
	Azadirachta indica	7.6±1.1[de]	Inefficient
25	*Eucalyptus camadulensis*	7.9±1.3[de]	Inefficient
	Cymbopogon citratus	7.6±1.1[e]	Inefficient
	Azadirachta indica	7.3±0.9[e]	Inefficient
12.5	*Eucalyptus camadulensis*	6.4±0.8[f]	Inefficient
	Cymbopogon citratus	6±0.0[f]	Inefficient
	Azadirachta indica	6±0.0[f]	Inefficient
6.25	*Eucalyptus camadulensis*	6±0.0[f]	Inefficient
	Cymbopogon citratus	6±0.0[f]	Inefficient
	Azadirachta indica	6±0.0[f]	Inefficient
3.12	*Eucalyptus camadulensis*	6±0.0[f]	Inefficient
	Cymbopogon citratus	6±0.0[f]	Inefficient
	Azadirachta indica	6±0.0[f]	Inefficient

a-f: Different letters indicate statistically relevant differences among inhibition diameters within each treatment using the Tukey test ($p \leq 0.05$).
Source: Authors

(MIC) and Minimal Bactericidal Concentrations (MBC) are shown in Table 8. Referring to survival rates, MBC/MIC ratios ≤ 2 were obtained with the copper sulphate, essential oil of *O. gratissimum* and aqueous extract of *E. camadulensis* confirming their antibacterial properties. However, we were unable to determine the MIC and MBC of *B. amyloliquefaciens*, *A. indica*, *C. citratus* and Proraly 50 EC due to their opacity.

Table 7. Survival rate (%) of *Xcc* colonies at different concentrations of substances tested.

Concentration	Copper hydroxide	Copper sulphate	*Bacillus amyloliquefaciens*	*Ocimum gratissimum*	Proraly 50EC	Neco 50EC	Azadirachta *indica*	*Cymbopogon citratus*	*Eucalyptus camadulenssis*
Survival rate%									
C1	-4.9±6.2^{a}	-5.7±3.8^{a}	9.7±1.71^{a}	-9.2±3.26^{a}	5±1.3^{a}	7.24±1.2^{a}	7.7±1.1^{a}	4.38±1.9^{a}	-2.8±1.8^{a}
C2	-0.6±6.7^{a}	6.3±4.64^{b}	30.5±6.43^{b}	1.3±2.99^{b}	19.3±3.2^{b}	26.01±4.4^{b}	43.8±11.2^{b}	38±11.2^{b}	5±1.76^{b}
C3	16.4±7.4^{b}	27.5±7.7^{c}	54.6±5.3^{c}	15.7±4.7^{c}	30.9±6.2^{c}	44.32±5.4^{c}	70.6±17.6^{c}	62.3±17.6^{C}	25.3±5.3^{c}
C4	37.2±13.9^{c}	49.7±10.8^{d}	75.1±4.12^{d}	30.9±4.5^{d}	67.2±10.5^{d}	76.7±9.4^{d}	90.5±23.1^{d}	89.1±24.3^{d}	40.3±7.2^{d}
C5	48.7±15.7^{cd}	64.3±16.4^{e}	96.4±5.6^{e}	37.9±4.4^{e}	90.9±3.4^{e}	100.6±8.9^{e}	109.1±29.2^{e}	99.3±26.3^{e}	64.8±9.5^{e}
C6	57.6±18.8^{d}	74.5±14.8^{e}	110±7.7^{f}	45.4±5.76^{f}	102.5±8.5^{f}	119.1±7.45^{f}	124.3±31.5^{f}	111.6±29.1^{f}	80.3±12.5^{f}
P-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

a-f: Different letters indicate statistically relevant differences among survival rates within each treatment using the Tukey test ($p < 0.05$).
Source : Authors

Table 8. Antibacterial parameters of the different substances.

Substance	Antibacterial parameter			
	MIC	MBC	MBC/MIC	Activity
Copper hydroxide (mg/ml)	12.5	12.5	1	Bactericidal
Copper sulphate (mg/ml)	12.5	25	2	Bactericidal
Ocimum gratissimum (µl/ml)	2.5	5	2	Bactericidal
Eucalyptus camadulensis (mg/ml)	50	100	2	Bactericidal

MIC: Minimum inhibitory concentration; MBC: minimum bactericidal concentration.
Source : Authors

Effect of treatments on the incidence and severity of citrus bacterial canker

The results showed that the averages of incidence and severity of CBC significantly differed among the treated substances. We noted a significant decrease of the incidence and severity on the treated plants compared to untreated controls, which incidence progressed from 41.32 to 56.12% after 120 days of observation (Figures 2 and 3). The C2 concentrations tested were the most effective. Analysis showed that the strongest disease reduction rates were obtained with copper hydroxide and sulfate at 12.5 mg/ml. However, with *O. gratissimum* at 10 µl/ml, we recorded a reduction in disease without significant difference with chemical products.

DISCUSSION

Through this study, we evaluated the antibacterial activity of the diverse substances *in vitro* and *in vivo* on the growth of *X. citri* pv. *citri*.

The two chemical products based on copper at 25 mg/ml, were the most active with the high inhibition diameters. Furthermore, the aqueous extract of *A. indica*, *C. citratus* and *E. camadulensis* and essential oils of *O. gratissimum* were each active inducing an inhibition diameter superior to 10 mm at the maximal tested concentrations. The lowest inhibition diameters were obtained with Neco 50EC and Proraly 50EC.

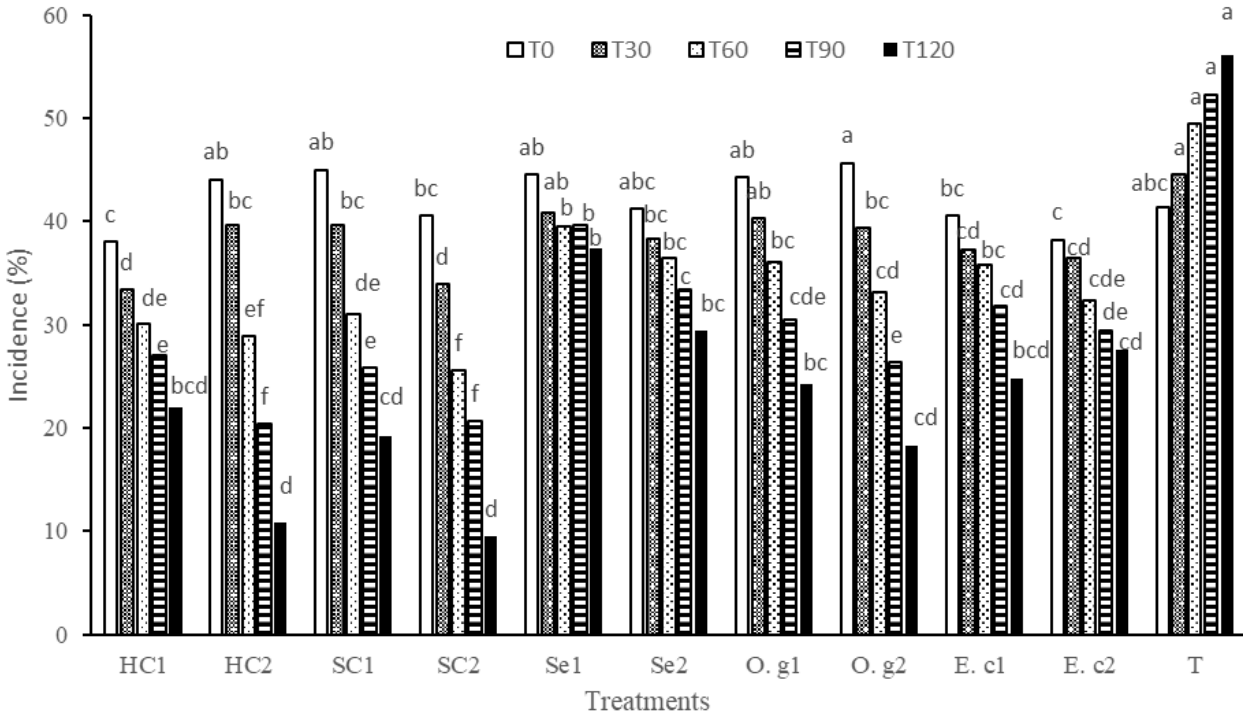

Figure 2. Disease incidence after treatment with different substances. **HC1 and HC2**: copper hydroxide at 25 and 12.5 mg/ml; **SC1 and SC2**: copper sulfate at 25 and 12.5 mg/ml; **Se1 and Se2**: *Bacillus amyloliquefaciens* formulation at 30 and 60 µl/ml; **O.g1 and O.g2**: He of *O. gratissimum* at 5 and 10 µl/ml; **E.c1 and E.c2**: Aqueous extract of *E. camadulensis* at 50 and 100 mg/ml; **T**: Control.
Source : Authors

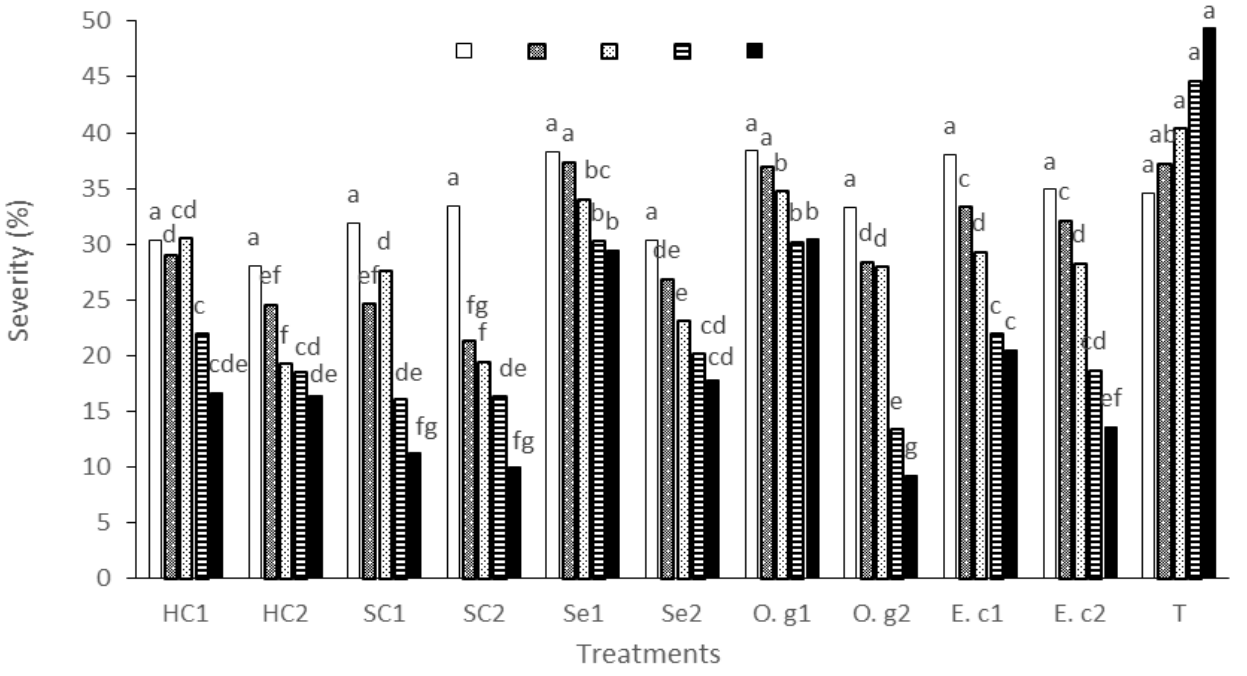

Figure 3. Disease severity after treatment with different substances. HC1 and HC2 : copper hydroxide at 25l and 12.5mg/ml; SC1 and SC2: copper sulfate at 25 and 12.5 mg/ml; Se1 and Se2: *Bacillus amyloliquefaciens* formulation at 30 and 60 µl/ml; O.g1 and O.g2: He of *O. gratissimum* at 5 and 10 µl/ml; E.c1 and E.c2: Aqueous extract of *E. camadulensis* at 50 and 100mg/ml; T: Control.
Source: Authors

According to MBC/MIC ratios of copper hydroxide (12.5 mg/ml), copper sulphate (25 mg/ml), *O. gratissimum* and *E. camadulensis* (50 mg/ml), the results showed that these substances have antibacterial properties.

For *in vivo* tests, the results showed that chemical products based on copper and the essential oil of *O. gratissimum* were the most effective, when they were used in foliar treatment each two week intervals.

In fact, the antibacterial action of copper hydroxide and copper sulphate *in vitro* and *in vivo* has been demonstrated by several studies (Girard, 2004; Idrissou-Touré et al., 2020; Behlau et al., 2021). However, some authors claimed that the use of copper can induce resistance to pathogens (Giraud et al., 2007; Villeneuve, 2008; Damien et al., 2017), create environmental pollution and copper accumulation in soils (Alva et al., 1995).

To attenuate the harmful effect of copper, some authors recommended its use in combination with mancozeb (Roberts et al., 2008; Fayette et al., 2012) or other biological components such as *B. subtilis* (Provost et al., 2012).

Studies have shown that the essential oil of *O. gratissimum* contains components such as thymol over 43%, gamma-terpinene 18.77% and para-cymene 6.77% (Soro et al., 2011), which would give it a bactericidal activity. Thus, thymol was one of the components of essential oils that are the most active against pathogens (Ajjouri et al., 2008).

The results confirmed those finding of Kpodekon et al. (2013) that demonstrated that the MICs and CMBs of *O. gratissimum* would be respectively between 6.10^{-3} and 144.10^{-3} mg/ml. In addition, Zombré et al. (2015) have shown that *O. gratissimum* was effective against bacterial blight of cashew and mango caused by *X. citri* pv. *mangiferaindica*. Saha et al. (2013) have also reported the antibacterial activity of *O. gratissimum* against Gram negative bacteria.

The effectiveness of aqueous plant extracts of *A. indica*, *C. citratus* and *E. camadulensis* has been proven in numerous studies, especially against fungi of the *Fusarium* genus (Dao, 2013; Tiendrebeogo, 2011).

As for the results obtained in the study with the biopesticide, the component of *B. amyloliquefaciens* would be the active molecule. Chen et al. (2009) reported that *B. amyloliquefaciens* produces various antibiotics which confer antibacterial activity. Roberts *et al*. (2008) and Ibrahim *et al.* (2016) have shown its efficacy on numerous species of *Xanthomonas*, *Pseudomonas* and *Erwinia*. Ye et al. (2016) reported that *in vivo* treatment with the combination of copper products and *B. subtilis* formulations reduced the incidence of CBC by only 19%, whereas untreated plants had 43%.

Conclusion

All the substances tested showed antibacterial properties against *Xcc* and their efficacy changes with concentration. The copper products were the most effective followed by the essential oil of *O. gratissimum*, the aqueous extracts of *E. camadulensis* and the substance formulation of *B. amyloliquefaciens* respectively. These substances were induced reductions of the incidence and severity of CBC, when applied at certain concentrations at two weeks intervals. Therefore, they can be tested in the field to determine their effectiveness.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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