EFFICACY OF COPPER ALTERNATIVES TO CONTROL CITRUS CANKER (*Xanthomonas citri* pv. *citri*) IN ACID LIME

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ABSTRACT

Citrus canker, caused by *Xanthomonas citri* pv. *citri*, is a widely occurring destructive bacterial disease of citrus, especially of acid lime (*Citrus aurantifolia*). Copper-based pesticides are widely used to control this disease, and their multiple and continuous applications not only cause toxicity to the plant but also induce resistance to bacteria. Therefore, this study was conducted to evaluate the efficacy of alternative antibiotics. The foliar spray was done to twelve-year-old acid lime variety Sunkagati-2 during July-September in 2023. Streptomycin sulphate + tetracycline hydrochloride, validamycin A, kasugamycin and zinkicide were compared with Bordeaux mixture and copper oxychloride. A randomized complete block design was assigned to three replications of each of the seven treatments. Bordeaux mixture had the least disease severity on leaves with a mean value of 11.75 ± 1.13%. Streptomycin sulphate + tetracycline hydrochloride, which had the second-lowest disease severity (14.96 ± 1.61%), was statistically similar to Bordeaux mixture. Similarly, validamycin A (21.54 ± 2.84%) and kasugamycin (21.75 ± 3.2%) also showed some level of effectiveness. Streptomycin sulphate + tetracycline hydrochloride showed the lowest size of lesion on leaves (3.09 ± 0.4 mm). The results of this study showed that streptomycin sulphate + tetracycline hydrochloride and validamycin A could be substituted for copper-based compounds. Further research on the effectiveness of these bactericides and their effect on plant toxicity and bacterial resistance is required to validate these results.

Key words : Acid lime, bactericides, citrus canker, copper alternatives, *Xanthomonas citri* pv. *citri*

INTRODUCTION

Citrus is an important fruit crop in Nepal, contributing 21.6% to overall fruit production. The total area of citrus cultivation was 49,306 ha in the year 2021-22, out of which 32,317 ha was a productive area with total production of 306,149 mt. Among citrus crops, acid lime has the second-highest area of cultivation (9,701 ha) with a productive area of 6,070 ha (MoALD, 2023). Citrus fruits have excellent nutritional and pharmacological value as they are considered to be a rich source of carbohydrates, vitamins, minerals, and dietary fibers. They also possess anti-cancer, antimicrobial, antioxidant, anti-inflammatory and hepatoprotective properties (Liu et al., 2012; Tomar et al., 2019). Considering its health, medicinal and industrial value, acid lime has great economic potential. However, several diseases in orchards, including powdery mildew, citrus canker, citrus greening, sooty mold, Phytophthora root rot and tristeza, have been limiting the growth of citrus. Among them, citrus canker, caused by *Xanthomonas citri* pv. *citri,* is a momentous disease of citrus crops, especially acid lime. The origin of this bacterial disease is controversial, but seen first in Asia (Das, 2003). Today canker is distributed globally. Symptoms of this disease can be seen on the leaves, twigs and fruits, which include water-soaked lesions usually surrounded by a yellow halo. Severely infested trees result in defoliation, twig dieback and premature fruit drops. In addition to that, the fruits that remain on trees lose market value (Behlau et al., 2010).

The application of copper-based bactericides has been found to be effective for the control of citrus canker in many experiments. However, a single application is not sufficient to suppress the disease severity on the surface of the leaf. In order to lower the amount of canker severity, multiple applications of copper at certain time intervals are needed (Shahbaz et al., 2023). The excess use of copper shows copper toxicity to the plant. Under such conditions, root morphology and plant nutrient composition are affected in many ways, including hindered root growth, decrease in chlorophyll content, necrosis, and yield decrease in young trees (Shabbir et al., 2020; Li et al., 2023; Hippler et al., 2018). In addition, multiple and wide uses of copper bactericides could develop bacterial strain resistance to copper. Copper-resistant *Xanthomonas* strains have been detected for the first time in Argentina (Behlau et al., 2011). Development of copper-resistant strains could impair the efficiency of copper-based bactericides. Formulation of nano-zinc oxide, which has very low phytotoxicity compared to copper, has reduced citrus canker in both leaves and fruits in grapefruit (Graham et al., 2016). Therefore, it is indispensable to evaluate the efficacy of bactericides, other than copper-based products, for the control of citrus canker. The purpose of this study was to evaluate and compare the efficacy of copper-based and non-copper-based bactericides in managing citrus canker under field conditions.

MATERIALS AND METHODS

The trial was conducted at National Citrus Research Programme (NCRP), Paripatle, Dhankuta, which is at 27^{o} 1” north latitude and 87^{0} 18” east longitude with an altitude of 1200 masl. Acid lime plants (var. Sunkagati-2) grown in the orchard of NCRP were sprayed with six different chemicals along with control (water spray) to manage citrus canker disease as shown in table 1.

Treatments were arranged in randomized complete block design (RCBD) with three replications of each of the seven treatments. There was one plant in one replication and there were a total of 21 plants. A total of four sprays, each at fifteen days interval, were applied during the month of July to September 2023 with the help of a knapsack sprayer (16 L capacity). Treatments were sprayed at 3 liters per tree with hollow-cone nozzles. The data were recorded after fifteen days of each spray. Disease scoring was done, and lesion diameter was measured from randomly selected five leaves from each of four directions of each tree. Additionally, the daily temperature and relative humidity of the research site were measured with the help of the Medhayantra sensor, which stores the data in cloud storage, and was extracted in MS Excel.

Disease scoring was done using a 0-4 visual assessment scale (Derso and Sijam, 2007).
Where,
0: No symptoms on leaves
1: 1-10% symptoms on leaves
2: 11-25% symptoms on leaves
3: 26-50% symptoms on leaves
4: Above 50% symptoms on leaves
Disease severity was calculated using the following formula as used by (Chester, 1950) :

$$\text{Disease severity (DS)} = \frac{\text{Sum of all disease score ratings}}{\text{Number of leaves observed} \times \text{Maximum rating scale}} \times 100$$

Area Under Disease Progress Curve (AUDPC) was calculated using the following formula (Das et al., 1992):

$$AUDPC = \sum_{i=1}^{n-1} [(X_{i+1} + X_i)/2][T_{i+1} - T_i]$$

Where,
X_i = disease severity on the i^{th} date
T_i = date on which disease was scored
n = number of dates on which disease was scored

The raw data was processed in MS Excel and subjected to arcsine transformation. Then the data were analyzed statistically using the analysis of variance (ANOVA) technique and the treatment means were separated by applying the least significant difference (LSD) test. The analysis was conducted using statistical software R (version 4.3.1) with add-on package agricolae.

Table 1. Details of different treatments sprayed in this experiment

Treatment	Chemical (Trade name)	Active ingredient (%)	Manufacturer	Dose per litre water
1	Plantomycin	Streptomycin sulphate (9%) + Tetracycline hydrochloride (1%) WP	Aries Agro Limited	0.2 g
2	Super Valida	Validamycin (10% SL)	Averstar Industries Co. Ltd	1.5 g
3	Kasu-B	Kasugamycin (3% SL)	Dhanuka Agritech Limited	2 ml
4	Bordeaux mixture	$Ca(OH)_2$: $CuSO_4$: water (1%)	-	10 g $Ca(OH)_2$ + 10 g $CuSO_4$
5	Curex	Copper oxychloride (50%WP)	Plant Remedies Private Limited	2 g
6	Zinkicide	Zinc oxide (200 ppm)	Trademark Nitrogen Corporation	3.5 ml
7	Control	Water spray	-	-

RESULTS AND DISCUSSION

Temperature values ranged from 22.8 ºC (6 Sept.) to 33.8 ºC (17 Sept.) during the study period with a mean temperature of 26 ºC. The highest relative humidity was recorded as 95% (7, 9, 16, 20 Aug., and 2 and 4 Sept.) while the lowest relative humidity was 76.2% (6 Sept.). The mean relative humidity was recorded as 93% during the study period (Fig. 1).

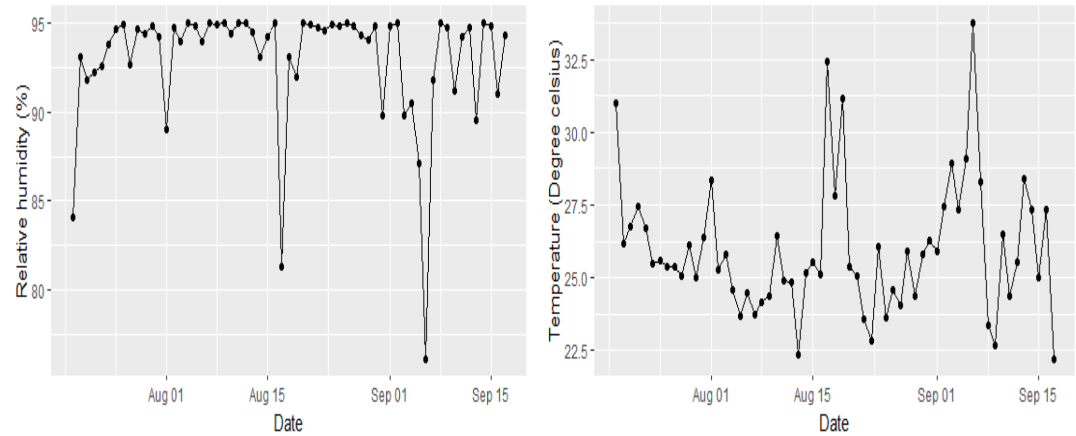

Fig. 1. Temperature (°C) and relative humidity (%) at NCRP, Dhankuta from July 19 to September 17, 2023

Table 2. Effect of different chemicals on citrus canker severity (%) on acid lime leaves var. Sunkagati-2 at National Citrus Research Programme, Paripatle, Dhankuta, Nepal from July to September 2023

Treatment	Disease severity (%)				
	Days after first application				
	15 days	30 days (Aug. 18)	45 days (Sept. 2)	60 days (Sept. 17)	Mean
Plantomycin (Streptomycin sulphate + tetracycline hydrochloride)	12.2^{d} ± 0.3 (20.41)	13.7^{ef} ± 1.3 (21.65)	20.3^{bcd} ± 2.7(26.56)	13.7^{c} ± 1.8 (21.6)	15.0^{c} ± 1.6 (22.67)
MU-REUM TAN (Validamycin)	20.3^{cd} ± 4.2 (26.56)	24.2^{cd} ± 2.7 (29.38)	24.2^{bcd} ± 3.4 (29.07)	17.5^{c} ± 0.0 (24.73)	21.5^{bc} ± 2.8 (27.53)
Kasu-B (Kasugamycin)	15.0^{d} ± 3.0 (22.58)	18.8^{de} ± 3.8 (25.5)	16.5^{cd} ± 2.8 (23.62)	36.7^{ab} ± 4.2 (37.21)	21.8^{bc} ± 3.2 (27.65)
Bordeaux mixture	13.7^{d} ± 1.4 (21.65)	9.8^{f} ± 1.8 (18.14)	13.7^{d} ± 2.6 (21.21)	9.8^{c} ± 2.3 (18.06)	11.8^{c} ± 1.1 (19.99)
Curex (Copper oxychloride)	43.3^{a} ± 4.2 (41.14)	50.0^{a} ± 2.5 (45)	30.8^{abc} ± 2.8 (33.59)	17.5^{c} ± 0.0 (24.73)	35.4^{a} ± 2.3 (36.49)
Zinkicide	27.5^{bc} ± 3.8 (31.52)	32.8^{bc} ± 6.9 (34.72)	36. 7^{ab} ± 3.3 (37.16)	32.8^{b} ± 6.7 (34.75)	32.5^{ab} ± 4.7 (34.6)
Control	32.8^{ab} ± 4.1(34.88)	36.7^{b} ± 3.2 (37.24)	43.3^{a} ± 3.4 (41.1)	45.2^{a} ± 4.7 (42.21)	39.6^{a} ± 4.5 (38.89)
Grand mean	23.6	26.6	26.5	24.7	25.3
F-test	***	***	*	***	***
CV (%)	25.3	23.4	37	24.9	24.8

CV: Coefficient of variation; *: Significant at 0.05 level of significance; ***: Significant at 0.001 level of significance; Values with the same letter in a column are not significantly different at 5% level of significance; Figures after ± indicates the standard error of mean (SEM) and figures in parenthesis indicate arcsine transformation.

There was a significant variation in the disease severity of canker among different treatments at all four observations (Table 2). The lowest disease severity (%) at 15 days was found in the plants sprayed with streptomycin sulphate 9% + tetracycline hydrochloride 1% WP (12.2 ± 0.3), followed by Bordeaux mixture 1% (13.7 ± 1.4) and kasugamycin 3% SL (15 ± 3). Disease severity (DS) at 30 days was lowest on citrus plants sprayed with Bordeaux mixture (13.7 ± 1.3), which was statistically similar to streptomycin sulphate 9% + tetracycline hydrochloride 1% WP (13.7 ± 1.3). The lowest disease severity at 45 days was on the plants sprayed with Bordeaux mixture (13.7 ± 2.6), followed by kasugamycin 3% SL (16.5 ± 2.79). Similarly, at 60 days, the lowest disease severity was found in plants sprayed with Bordeaux mixture (9.8 ± 2.3), followed by streptomycin sulphate 9% + tetracycline hydrochloride 1% WP (13.7 ± 1.8). The mean disease severity ranged from 11.8 to 39.6 with an average of 25.3. The highest mean disease severity was recorded in the plants sprayed with water (39.6 ± 4.5) and the plants sprayed with copper oxychloride 50% WP had the second highest canker severity on leaves (35.4 ± 2.3), whereas the least severity was seen in plants sprayed with Bordeaux mixture (11.8 ± 1.1) followed by streptomycin sulphate 9% + tetracycline hydrochloride 1% WP (15 ± 1.6) sprayed plants.

The area under disease progress curve (AUDPC) was found statistically different among the tested chemicals. The AUDPC values ranged from 528.8 to 1785 with a mean value of 1158.2. The highest AUDPC was recorded in untreated control (1785 ± 205), which was not significantly different with plants treated with copper oxychloride 50% WP (1668.8 ± 126.4). On the other hand, the lowest AUDPC value was observed in citrus plants sprayed with Bordeaux mixture 1% (528.8 ± 73.3), followed by streptomycin sulphate 9% + tetracycline hydrochloride 1% WP (703.7 ± 96.4) and kasugamycin 10% SL (917.5 ± 170.3). Among the tested chemicals, the size of lesions on the leaves was not found statistically different. The size of the lesion diameter ranged from 3.1 mm to 4.3 mm.
The results of this study revealed that the application of Bordeaux mixture was most effective in controlling canker in acid lime var. Sunkagati-2. Besides that, streptomycin sulphate 9% + Tetracycline hydrochloride 1% WP was also excellent in reducing the severity of canker in leaves. Further, other treatments validamycin A and kasugamycin also showed satisfactory results against the citrus canker on leaves.

Table 3. Effect of different chemicals on area under disease progress curve (AUDPC) and lesion diameter of citrus canker on acid lime leaves var. Sunkagati-2 at National Citrus Research Programme, Paripatle, Dhankuta, Nepal from July to September 2023

Treatment	AUDPC (%)	Lesion diameter (mm)
Plantomycin (streptomycin sulphate + tetracycline hydrochloride)	$703.8^{c} \pm 96.4$	$3.1^{a} \pm 0.4$
MU-REUM TAN (validamycin A)	$1008.8^{bc} \pm 167.4$	$3.5^{a} \pm 0.5$
Kasu-B (kasugamycin)	$917.5^{c} \pm 170.3$	$3.3^{a} \pm 0.3$
Bordeaux mixture	$528.8^{c} \pm 73.3$	$3.1^{a} \pm 0.6$
Curex (copper oxychloride)	$1668.8^{a} \pm 126.4$	$4.2^{a} \pm 0.3$

Treatment	AUDPC (%)	Lesion diameter (mm)
Zinkicide	1495.0[ab] ± 254.0	3.5[a] ± 0.5
Control	1785.0[a] ± 205.0	4.3[a] ± 0.1
Grand mean	1158.2	3.6
F-test	**	NS
CV (%)	25.9	21.6

CV: Coefficient of variation; **: Significant at 0.01 level of significance; Values with the same letter in a column are not significantly different at 5% level of significance; Figures after ± indicates the standard error of mean (SEM); NS: Not significant

Bordeaux mixture, a copper-based fungicide and bactericide, performed best for reducing severity of citrus canker disease. Unlike Bordeaux mixture, another copper-based bactericide - copper oxychloride - had the highest disease severity after untreated control. However, there was a great decline in disease severity in subsequent observations after the first observation (Table 2). Many researchers have reported copper bactericides are effective in reducing canker disease (Behlau et al., 2008; Dhakal et al., 1970; Rehman et al., 2020). As this study was to evaluate the effectiveness of non-copper bactericides against the citrus canker in leaves, streptomycin sulphate + tetracycline hydrochloride followed by validamycin A and kasugamycin showed promising results. These results are similar with the findings of Graham et al. (2008), who evaluated streptomycin in controlling citrus canker of sweet orange in Brazil. They reported low disease severity of canker (25.8%) with the spray of streptomycin sulphate. Validamycin and kasugamycin are not evaluated much for the control of citrus canker. Thus, streptomycin sulphate + tetracycline hydrochloride, validamycin and kasugamycin show potential as antibiotics to substitute copper-based antibiotics for the control of citrus canker.

CONCLUSIONS

Among all the treatments, Bordeaux mixture performed the best in the reduction of disease severity of citrus canker on the leaves of acid lime var. Sunkagati-2. However, streptomycin sulphate + tetracycline hydrochloride was most effective in reducing citrus canker lesion diameter in leaves. Streptomycin sulphate + tetracycline hydrochloride was also effective in reducing the disease severity. Further, other non-copper-based bactericides like validamycin A and kasugamycin also did well to control citrus canker in leaves as compared to untreated control. Therefore, streptomycin sulphate + tetracycline hydrochloride (Plantomycin), validamycin A and kasugamycin could be used as alternative chemicals for the control of citrus canker in acid lime. However, the environmental effect and phytotoxicity of these bactericides should be evaluated before making a final recommendation.

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