International Journal of Scientific Research in Engineering and Management (IJSREM)
Volume: 09 Issue: 04 | April - 2025 SJIF Rating: 8.586 ISSN: 2582-3930

Efficacy of Chemicals Against *Xanthomonas Axonopodis Pv. Citri* Under In-Vitro Condition

Sijan Lamsal, Sudeep Khanal, Sandipa Dhakal

Abstract

Citrus canker, caused by the bacterium *Xanthomonas axonopodis pv. citri*, is one of the most devastating diseases hampering worldwide citrus production, including in Nepal. Citrus canker is a significant threat to all citrus-growing regions because of its rapid spread, high potential for damage, and impact on export sales and domestic trade. The study was to solve the major problem associated with citrus canker by identifying and evaluating the in vitro efficacy of different chemicals available in the local market. The pathogen was isolated from the diseased plants, and a pure culture was obtained. In vitro efficacy of streptomycin, Bordeaux mixture, kasugamycin, and copper oxychloride was assessed by paper disc technique. The efficacy of different chemicals was compared based on the formation of a zone of inhibition (ZOI). After 48 hours of inoculation, diameters of zones of inhibition were measured in each, and statistical analysis was performed. Streptomycin (100 ppm) was statistically more significant than all other treatments and formed the highest zone of inhibition, followed by kasugamycin (100 ppm). Copper oxychloride (0.3%) and Bordeaux mixture (1.5%) were least effective and found statistically at par. The results revealed that there was a significant difference between different chemicals inhibiting the growth of *Xanthomonas axonopodis pv. citri*. The study recommends the use of streptomycin (100 ppm) among the chemicals for effective management of the disease.

Keywords: Citrus canker, Streptomycin, *Xanthomonas axonopodis*, Zone of inhibition

1. INTRODUCTION

Citrus fruits are rich in nutrients and minerals and are popular for their refreshing value. It is considered to be the best source of vitamin C, sugars, amino acids, and other nutrients. Nepal is one of the centres of citrus diversity, and many of its species are grown in this country. The total area under citrus production and the productivity of citrus is 46,412 hectares, 271,908 mt, and 9.57 mt per hectare (MOALD, 2018/19). The mid-hill region, which accounts for about 1.5 million ha, is quite suitable for citrus cultivation (Shrestha & Verma, 1999).

Xanthomonas are obligatory aerobic, Gram-negative bacteria, and they consist of a single polar flagellum. They are mesophilic bacteria having an optimum temperature range of 28-30°C and thrive up to a maximum temperature of 35-39°C (Mehrotra, 1998). On leaves, stems, and fruit, the pathogen causes distinctive necrotic, erumpent lesions. Severe infections can result in defoliation, blemished fruit, premature fruit drop, twig dieback, and general tree decline. Lesions get ruptured in the leaf as it matures and create crater-like spots normally surrounded by oily and water-soaked margins with a yellow halo, and often old lesions fall out, leaving only round holes, but in fruits the lesions are only deep in the skin and appear corky or scabby (USDA, 1997). The presence of citrus leaf miner greatly exacerbates citrus canker incidence and severity (Gottwald et al., 2002) as its feeding activity provides entry points for the bacterium (Belasque et al., 2005). With the help of air currents, insects, birds, humans, and rain, citrus cankers are spread over short distances. Splashing rain and winds in excess of 8.0 m/s can disperse the pathogen (Gottwald et al., 1989). Rainfall of 1000 mm or more per year is also a conducive environment for the pathogen (Verniere et al., 2003). *Xanthomonas axonopodis pv. citri* easily persists season to season in old lesions, especially in warmer climates and in lesions formed late in the growing season (Pruvost et al., 2002).

Of all the agricultural pests and diseases that threaten lime, citrus canker is one of the most devastating. Because of its rapid spread, high potential for damage, and impact on export sales and domestic trade, citrus canker is a significant threat to all citrus-growing regions. Canker A, or Asiatic canker, caused by X. axonopodispv.citri (Xac), is the most destructive form among all the strains (Gottwald and Graham, 2000). Citrus canker is the endemic disease in many tropical and subtropical citrus-growing areas (Goto, 1992). 55% of the area of citrus orchards in the USA was lost during 1996 and 2008, and approximately 50 million dollars per year are spent for management of this disease (USDA, 2014). The experience from other citrus-producing countries with endemic canker indicates if the incidence of infected fruit in a block is greater than 2% to 5%, it is hazardous to harvest fruit from that block for the fresh market (Ritenour et al., 2007). There is not any effective means of disease management, and most of the time we have to rely on cultural practices, copper-based compounds, and some antibiotics, which are not enough. This research will assist in solving the major problem associated with citrus canker by identifying a suitable chemical, which is much more effective. Cost-effective treatment with high efficacy will certainly be adopted. It also opens the door to start the research activities related to agrochemicals. Agrochemicals are noxious for the environment (Huang, 1997). Therefore, concentration is focused on a propitious method of disease management that will be friendly for the environment as well as for mankind (Sutton, 1996).

The purpose of the study was to evaluate the in vitro efficacy of different chemicals available in the local market.

2. MATERIALS AND METHOD

This experiment was carried out in the central laboratory of the Institute of Agriculture and Animal Science, Lamjung Campus, Sundarbazar, during November 2019. A plant with typical symptoms of citrus canker was identified. Affected samples of leaves were collected from Sundarbazar, Lamjung, and taken to the laboratory. The study was designed in a complete randomized design (CRD) with 5 treatments and 6 replications. Four chemicals, viz., streptomycin (100 ppm), Bordeaux mixture (1.5%), kasugamycin (100 ppm), and copper oxychloride (0.3%), were evaluated against *Xanthomonas axonopodis pv. citri* under in vitro conditions by paper disc technique. All the isolation and inoculation work was carried out in a laminar flow under an aseptic condition. Laminar flow was sterilized through UV light exposure for 20 minutes followed by application of 70% ethanol, and the materials such as petri plates, beakers, spatulas, test tubes, forceps, media, and distilled water were sterilized in an autoclave at 121°C and 15 psi for 15-20 minutes.

Nutrient agar was used as the nutrient media for the growth of bacteria. Bacteria from the affected sample was isolated under aseptic conditions and was transferred to a freshly prepared nutrient agar (NA) plate and was incubated at 28°C for 48 hours. In order to get a pure colony, individual bacterial colonies having smooth margins, yellowish coloration, and offensive smells were picked and restreaked on another NA plate with the help of a sterile loop, and 48-hour-old cultures were made again. Those cultures were dissolved in 20% sterile glycerol made in SDW and were stored at -4°C for future use. Pure cultures that were incubated for 2 days were used for the preparation of mother culture for further multiplication and inoculum preparation. The stock solution of each chemical to be used was made and diluted to get the required concentration. Mathematical calculation were made with the help of dilution formula, i.e., $C_1V_1 = C_2V_2$

The bacterial suspension was prepared from a 48-hr-old culture on NA by pouring 20 ml of sterilized water on the colony surface in a petri plate and gently scraping with the help of a sterilized needle and then shaking until all the bacterial mass gets dissolved uniformly. About 15-20 ml of nutrient agar (NA) was poured in sterilized Petri plates and allowed to solidify. 100 µl of bacterial solution was measured by using a micropipette and then dispersed on an NA plate and spread evenly over the surface by using a sterile bent glass rod to form the bacterial mat. Four pieces of paper disc (blotting paper), each having a 6 mm diameter, were kept over the bacterial mat at equidistance. 4 µl of a particular treatment was applied to each paper disc by using a micropipette, and replication was made. The same process was repeated for other treatments as well. Prepared Petri dishes were sealed with Parafilm tape and put in an incubator for two days at 28°C. After 48 hours, petri plates were observed for the data collection.

The inhibition of the growth of bacteria around the treated paper disc formed the halo, which was termed as the zone of inhibition (ZOI). Average diameters of each ZOI were measured by using a linear scale and recording. The real diameter of inhibition was calculated by subtracting the size of the paper disc, which was 6 mm. The data obtained were entered in MS Excel (2013), and results were analyzed by using R-stat software.

3. RESULTS AND DISCUSSION

Results

The in vitro evaluation of different chemicals was assessed by the paper disc method, and results are presented in Table 1.

All the tested chemicals exhibited more or less inhibitory action against the tested pathogen under in vitro conditions. In vitro antibacterial effects were determined by measuring the zone of inhibition (ZOI) with the help of a linear scale. Streptomycin (100 ppm) showed the highest ZOI with 34.43 mm of diameter, which was statistically significant compared to all other treatments, followed by Kasugamycin (12.82 mm). Bordeaux mixture (1.19 mm) and copper oxychloride (1.38 mm) were least effective and are at par.

The results revealed that there was a significant difference between different chemicals inhibiting the growth of *Xanthomonas axonopodis pv. citri.*

Table 1: Effect of Treatment on Growth Inhibition

Treatments	Actual diameter of Zone of Inhibition in mm (mean ± S.E)
Streptomycin (100 ppm)	34.43 ± 6.77 [a]
Bordeaux Mixture (1.5%)	1.19 ± 0.60 [c]
Kasugamycin (100 ppm)	12.82 ± 2.73 [b]
Copper Oxychloride (0.3%)	1.38 ± 1.06 [c]
Significance	***
P value	0.00
Grand Mean	9.96
LSD	9.65

***: Significant at 0.01 level of significance

LSD: Least significant difference

Data with the same alphabet are not, and data with different alphabets are found to be significantly different.

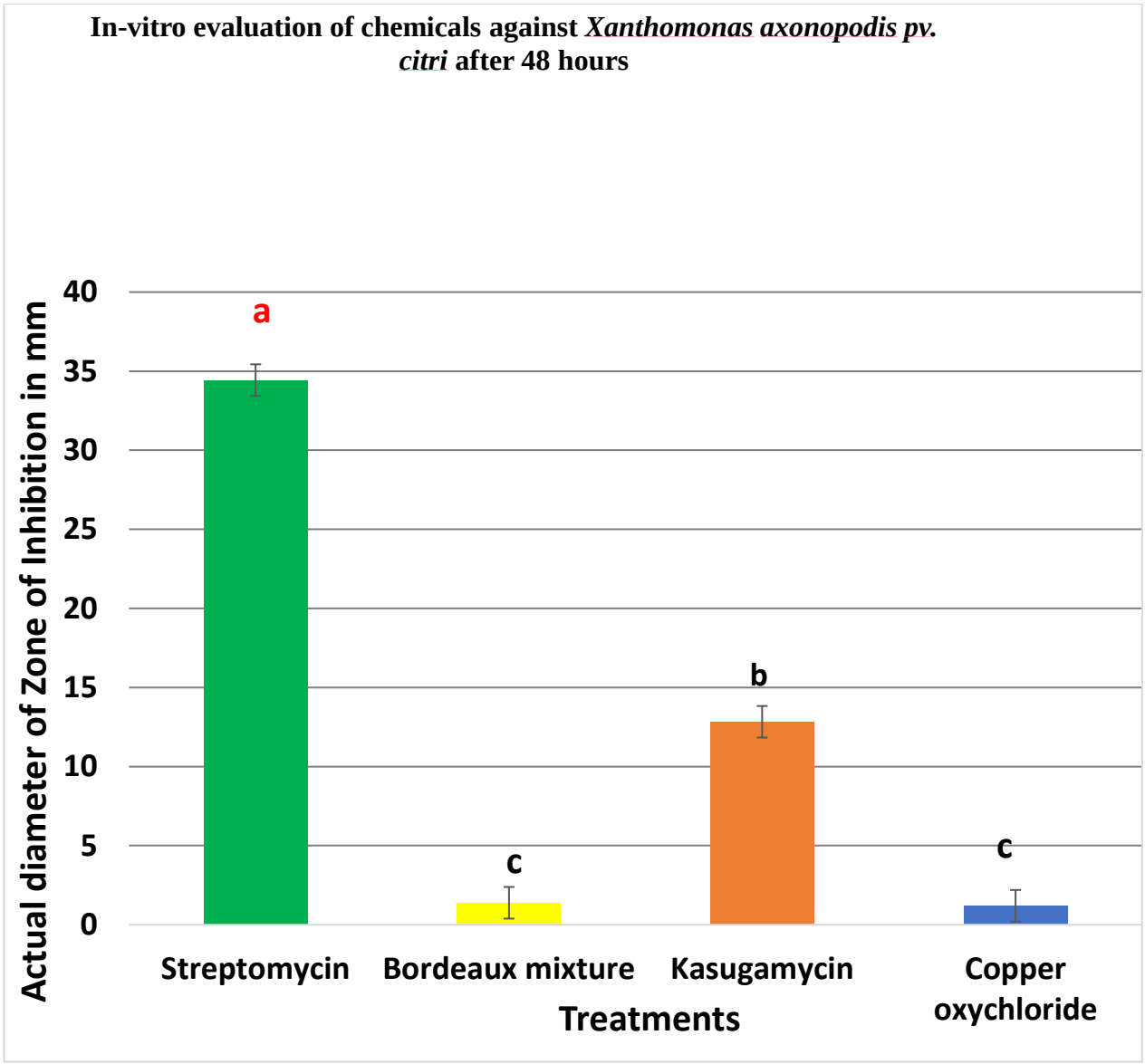

Figure 1: In vitro effect of chemicals against *Xanthomonas axonopodis pv. citri.*

Different alphabet above the bar shows their statistical differences

Discussion

The result of streptomycin being effective could be seen supported by the findings of different researchers. A similar result was recorded in the findings of Khan et al. (1992). Shahid et al. (2005) reported that streptomycin significantly inhibited the multiplication of the *Xanthomonas axonopodis pv. citri* at all concentrations. (Each at 0.01, 0.1, and 1% concentrations). Streptomycin binds irreversibly to bacterial ribosomes and thereby inhibits protein synthesis (McManus & Stockwell, 2001).

Jadhav et al. (2018) reported Kasugamycin (100 ppm) as effective against *Xanthomonas axonopodis pv. citri.* Kasugamycin is a bacterial metabolite, a protein synthesis inhibitor, and an antifungal agrochemical.

Copper oxychloride and Bordeaux mixture were least effective (El-Goorani, 1989). In our study, Bordeaux mixture and copper oxychloride were found to be the least effective; however, Jadhav et al. (2018) reported satisfactory results of

copper oxychloride and Bordeaux mixture at 1000 ppm and 2000 ppm. This might be due to the presence of multiple races of bacteria, and different races of bacteria respond differently to respective chemicals.

Copper ions present in Bordeaux mixture are deposited in pathogenic cells, affecting them toxically and preventing pathogenic spore germination. Copper oxychloride has multi-site activity, and absorbed copper disrupts the enzyme systems of pathogens.

4. CONCLUSION

Streptomycin (100 ppm) was found to be the most effective among tested chemicals, followed by Kasugamycin (100 ppm). Copper oxychloride (0.3%) and Bordeaux mixture (1.5%) were least effective.

Chemicals tested above in this study for in-vitro inhibition of bacterial growth could be the better substitute under field conditions for the management of citrus canker. Concentration is focused on a propitious method of disease management that will be friendly for the environment as well as for mankind.

ACKNOWLEDGEMENT

The author extends his profound gratitude and appreciation to the Institute of Agriculture and Animal Science, Lamjung Campus, for providing laboratory facilities.

References

Belasque Jr, J., A. Parra-Pedrazzoli, J. Rodrigues Neto, P. Yamamoto, M. Chagas, J. Parra & J. Hartung. 2005. Adult citrus leafminers (*Phyllocnistis citrella*) are not efficient vectors for *Xanthomonas axonopodis pv. citri.* Plant Disease. 89(6): 590-594.

Goto, M. 1992. Citrus canker. In 'Plant diseases of international importance. Vol. III. Diseases of fruit crops'.(Eds J Kumar, HS Chaube, US Singh, AN Mukhopadhyay). 250-269.

Gottwald, T., X. Sun, T. Riley, J. Graham, F. Ferrandino & E. Taylor. 2002. Geo-referenced spatiotemporal analysis of the urban citrus canker epidemic in Florida. Phytopathology. 92(4), pp. 361-377.

Gottwald, T., L. Timmer & R. McGuire. 1989. Analysis of disease progress of Citrus canker in nurseries in Argentina. Phytopathology. 79(11): 1276-1283.

Gottwald, T. R. and Graham, J. H. 2000. Bacterial Diseases. In: Compendium of Citrus Diseases, 2nd ed. by L. W. Timmer, S. M. Garnsey and J. H. Graham, pp. 5−8. American Phytopathological Society Press, St. Paul, MN.

Huang, H. 1997. Biological control of soilborne diseases in Canada. In International Symposium on Clean Agriculture, Sapporo: OECD: 52-59.

Jadhav, R., A. More, K. Apet & B. Dandnaik. 2018. In Vitro Bioefficacy of Different Antibiotics, Fungicides and Botanicals against (*Xanthomonas axonopodis pv. citri*) Causing Bacterial Canker of Kagzi Lime. International Journal of Current Microbiology and Applied Sciences. 6: 1693-1699.

Khan, M. M., M. A. Khan, M. Haq, M & N. Javed. 1992. Antibacterial activity of various toxicants against *Xanthomonas campestris pv. citri* for the control of citrus canker disease. In Proceedings of the First International Seminar on Citriculture in Pakistan. 2-5.

McManus, P. & V. Stockwell. 2001. Antibiotic use for plant disease management in the United States. Plant Health Progress. 2(1): 14.

Mehrotra, R. 1998. Plant Pathology. Tata MC Grow Hill Pub. Co. Ltd., New Delhi.

MOALD. 2075/76. Statistical information on Nepalese Agriculture. Ministry of Agriculture and Livestock Development. Kathmandu, Nepal. 171

Pruvost, O., B. Boher, C. Brocherieux, M. Nicole & F. Chiroleu. 2002. Survival of *Xanthomonas axonopodis pv. citri* in Leaf Lesions Under Tropical Environmental Conditions and Simulated Splash Dispersal of Inoculum. Phytopathology 92: 336-346.

Ritenour, M., J. Graham, R. Muraro, and H. Browning. 2007. Florida citrus growers and packers visit Argentina's fresh fruit operations. Citrus Ind. 88(11):1–12.

Shahid, M., S. Sahi, M. Ghazanfar, M. Inam, I. Haq, Y. Haq, T. Ahmad. 2005. Evaluation of different toxicants against *Xanthomonas campestris pv. citri* (Hasse) Dows. Int. J. Agri. Biol. 7(1): 121-124.

Shrestha, P. P. & S. K. Verma. 1999. Development and outlook of citrus industry in Nepal. In Proceedings of national Horticulture workshop. 48-57.

Sutton, T. 1996. Changing options for the control of deciduous fruit tree diseases. Annual review of phytopathology. 34(1): 527-547.

Verniere, C., T. Gottwald & O. Pruvost. 2003. Disease development and symptom expression of *Xanthomonas axonopodis pv. citri* in various citrus plant tissues. Phytopathology. 93(7): 832-843.