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# Potential of *Levofloxacin and Ciprofloxacin* against citrus canker caused by *Xanthomonas axonopodis* pv*. citri*

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## Abstract

Citrus canker caused by *Xanthomonas axonopodis* pv. *citri* (*Xac*) is the most devastating threat to the world citrus industry. A greenhouse and field trail were conducted at Department of Plant Pathology, University of Agriculture Faisalabad. Levofloxacin, Ciprofloxacin were tested against *Xac* by injection technique under Randomized Complete Block Design (RCBD). Three concentrations (1, 1.5 and 2 %) after 10, 20, and 30 days were tested. Out of these treatments, combination of Levofloxacin and Ciprofloxacin (14.51 %) followed by Levofloxacin 17.48 %, Ciprofloxacin (21.66 %), in greenhouse respectively. In interaction between treatments and concentrations, combination of Levofloxacin and Ciprofloxacin expressed minimum disease incidence (10.44 %) at 2 % and 9.278 % after 30 days of inoculation in the interaction between treatments and days. In field study, combination of Levofloxacin and Ciprofloxacin (17.51 %) followed by Levofloxacin 20.48 %, Ciprofloxacin (24.09 %), and control (64.00 %) respectively. Interaction between treatments and concentrations, combination of Levofloxacin and Ciprofloxacin expressed minimum disease incidence (13.44 %) at 2 % while in interaction between treatments and days, this combination showed (12.27 %) disease incidence followed by Levofloxacin 15.27 % and Ciprofloxacin 17.22 % and control 69.11 % after 30 days of inoculation. In conclusion, combination of Ciprofloxacin and Levofloxacin expressed best results at concentration of 2 % after 30 days of inoculation.

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## Introduction

Citrus belongs to family *Rutaceae* and is known for its versatility and unique property of having multiple fruit species among its rank making it an important crop all over the world including Pakistan (Wali *et al.*, 2013). Among biotic factors various bacterial diseases causing massive losses in which citrus canker caused by *Xanthomonas axonopodis* pv. *citri (Xac)* is the most damaging one. Citrus canker signifies as most devastating threat to citrus and cause severe losses in the world (Ware, 2015). In United States, more than 13 million US dollars are dedicated annually to citrus canker control program (Zhang, 2011). *Xac* is a straight rod shaped, mono-flagellum, gram negative bacteria which give yellowish colonial growth due to production of xanthomonadin pigment. The citrus canker produces typical necrotic lesions on stem, leaves and fruits. The disease causes leaf fall, drying of twigs and premature fruit drop which leads to low quality fruits and yield losses (Gottwald *et al.,* 2002; Graham *et al.,* 2004). One of the key strategies being used in management of disease is use of resistant verities. Use of such disease resistant verities is of great help in disease management and saving losses (Deng *et al.,* 2010) but this comes with additional constraints including a long term durational span required for cross breeding and checking of each generation separately, attaining of additional traits which are of no good in disease management and probably exerts fitness penalty reducing plant yields. Over the time, canker disease has been tried to be managed by use of various synthetic chemicals.

These chemicals have been somewhat effective in disease control, but their side effects outweigh their benefits. They cause massive water, air, and soil contamination as a large portion of these chemicals either get mixed up with runoff or rainwater or disposed away by the air (Heinlaan *et al.,* 2008). Pesticides also causes various level of toxic effects on every population group with manufacturing workers, agricultural farmers and people living in close vicinity. Long term low concentration absorbance of such chemicals by human results into endocrine functional abnormalities; which includes role in production and destruction of hormones, dementia, reproductive abnormalities and certain forms of cancers (Mostafalou and Abdollahi, 2013). These chemicals also cause severe damage to beneficial soil microbiome resulting in reduction of soil biodiversity. For management of bacterial diseases various bactericides having copper as an active ingredient has been used. Use of such chemicals is effective in control of disease to an extent but it also causes some severe side effects, in many cases continuous application of copper has been seen to cause accumulation of copper residues on to top layer of soil bounded with organic matter which implies possibility of toxic impact. Also traces of copper left on to plant products later consumed by humans can result in to excess of copper in body which can produce various harmful effects including free radical production, per-oxidation of lipids, and antioxidant imbalance (Husak, 2015). In consideration to the above facts, its need of time to devise a strategy that does not involve the use of such chemicals and does not pollute the environment is essential. In response to this need, use of antibiotics can be a valuable asset in controlling the disease. These remain active on plants for less than a week and have no considerable residues on harvested fruits. Various experiments have been done to check the efficacy of certain antibiotics against the *Xac* which revealed that use of antibiotics is relatively more than the traditional pesticides as it does not produce any harmful residual effects. The antibiotics have been observed massively to reduce pathogen growth but investigation on new antibiotics at optimum concentration is the need of hour. So that establishment of resistance against *Xac* in already available antibiotics should be addressed (Mubeen *et al.,* 2015). Therefore, the current study was planned to evaluate the efficacy of certain antibiotics which are available in the local market.

## Materials and methods

The assessment of three concentrations (1, 1.5 and 2 %) of both antibiotics and their combination (Table 1) and control (distilled water) were conducted in greenhouse and field by using injection and spray method.

For obtaining these concentrations, an ample quantity of stock solutions of each antibiotic was prepared by following protocol explained by Rehman *et al,* 2015. Leaves showing typical characteristics canker symptoms were collected in brown paper bags (10" x 12") then taken to Phyto-bacteriology Lab. for isolation of *Xac* by using streaking method (Ruangpan and Tendencia, 2004). Firstly, Nutrient Agar (NA) media (Beef extract 3g, Glucose 2.5g, Agar 15g and Peptone 5g, and 1000 ml distilled water for 1-liter media) was prepared and autoclaved (RTA85, Robus United Kingdom) at 121ºC temperature and 15 psi pressure for 15 minutes (Hemraj *et al*, 2013). Small infected areas of leaves along with healthy portions (5-7 mm long pieces) were cut into small pieces and surface sterilized them with 70 % ethanol and then washed three times in sterile water and dried them on filter paper to avoid the contamination.

These pieces were transferred on NA media containing petri plates using sterilized forceps and after wrapping and labelling with permanent marker, incubated (temperature controlled with range ±2 ºC ambient to 70 ºC) at 25°C for 24 hours (Riaz *et al.,* 2008). Bacterial growth in the form of yellow ooze was appeared beneath the infected samples after 24-36 hours and transferred them to new plates containing NA media by using streak method for purification. Round colonies along with yellow color were appeared after 24-36 hours incubation period. Bacterial inoculum was prepared in broth media by putting purified colonies in it and kept on shaker for overnight. In greenhouse experiment, antibiotics (Ciprofloxin & Levofloxacin) were used at the concentrations of 1, 1.5 and 2 % and were applied on one-year old citrus plants grown in pots @ 10 mL per liter which were already inoculated with 10 mL suspension of *Xac* (Jahanshir and Dzhalilov, 2010).

Three replications of each treatment and concentrations were used. Plants which were considered as control treated with sterile distilled water. Three replications were maintained. Data were calculated following RCBD after 10, 20 and 30-days interval.

One-year old plants of susceptible citrus plants (Grapefruit) were transplanted into established field and *Xac* suspension was sprayed. The antibiotics with different concentrations @ 10 mL/liter were applied by spraying method. Treatments were applied to the plants arranged by following (RCBD) Randomized Complete Block Design Data on canker incidence were recorded after 10, 20- and 30-days interval.

## Results

All treatments (T), concentration (C), days (D) and their interactions (TxC), (TxD) except (CxD) and (TxCxD) expressed significant effects against citrus canker. Lowest disease incidence (14.51%) was observed under the combination of Levofloxacin + Ciprofloxacin, followed by Levofloxacin (17.48 %) and Ciprofloxacin (21.66 %) as compared to the control (Table 2 & Fig.1).

**Table 1.** Detail list of antibiotics, their active ingredients, molecular formula and molecular weight used in this study.

| Sr No. | Antibiotics | Active ingredients | Molecular formula | Molecular weight |
|---|---|---|---|---|
| 1 | Levofloxacin | Levaquin | $C_{18}H_{20}FN_3O_4$ | 370.38 g/mol |
| 2 | Ciprofloxacin | Ciprofloxacin hydrochloride | $C_{17}H_{18}FN_3O_3$ | 331.346 g/mol |
| 3 | Water | Control | $H_2O$ | 18.01528 g/mol |

In treatments and concentration interaction (TxC), Ciprofloxacin expressed 17.38, 21.66 and 24.22, Levofloxacin 13.33, 17.88, 21.22 percent disease incidence at 1, 1.5, 2 % concentrations while minimum disease incidence was observed by the combination of both (Levofloxacin and Ciprofloxacin) 10.44, 14.88 and 18.22 % respectively at three concentrations as compared to the control (Table 3 & Fig. 2). Interaction between treatment and days (TxD) exhibited that Ciprofloxin expressed (27.33, 21.72 and 14.22) % disease incidence while Levofloxacin (23.05, 17.11 and 12.27) and Levofloxacin + Ciprofloxacin 20.16, 14.11 and 9.27 % disease incidence when applied @ 1, 1.5 and 2 % after ten, twenty and thirty days consistently as compared to the control (Table 4 & Fig. 3).

**Table 2.** Evaluation of *Levofloxacin* and *Ciprofloxacin* against citrus canker under greenhouse conditions.

| Sr # | Treatments | Disease incidence (%) |
|---|---|---|
| $T_1$ | Ciprofloxin | 21.66b |
| $T_2$ | Levofloxacin | 17.48c |
| $T_3$ | Levofloxacin + Ciprofloxin | 14.51d |
| $T_4$ | Control | 60.00a |
| | LSD | 1.3658 |

Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$).

**Table 3.** Impact of interaction between treatments and concentrations (T×C) against citrus canker under greenhouse conditions.

| Sr # | Treatments | Disease incidence (%) | | |
|---|---|---|---|---|
| | | Concentrations (%) | | |
| | | At 1 % | At 1.5 % | At 2 % |
| $T_1$ | Ciprofloxin | 24.22c | 21.66d | 17.38e |
| $T_2$ | Levofloxacin | 21.22d | 17.88e | 13.33f |
| $T_3$ | Levofloxacin + Ciprofloxin | 18.22e | 14.88f | 10.44g |
| $T_4$ | Control | 54.00b | 58.00a | 58.00a |
| | LSD | | 2.3657 | |

Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$).

*Evaluation of antibiotics against citrus canker under field conditions*

All treatments (T), concentration (C), days (D) their interactions (T×C), (T×D) except (C×D) and (T×C×D) showed significant effects against citrus canker. Least disease incidence (17.51) was expressed in the combination of Levofloxacin + Ciprofloxin, followed by Levofloxacin (20.48) % and Ciprofloxin (24.09) as compared to the control (Table 5 & Fig. 4).

**Table 4.** Impact of interaction between treatments and days (T×D) against citrus canker under greenhouse conditions.

| Sr # | Treatments | Disease incidence (%) | | |
|---|---|---|---|---|
| | | Days | | |
| | | After 10 days | After 20 days | After 30 days |
| $T_1$ | Ciprofloxin | 27.333d | 21.722ef | 14.222h |
| $T_2$ | Levofloxacin | 23.056e | 17.111g | 12.278h |
| $T_3$ | Levofloxacin + Ciprofloxin | 20.167f | 14.111h | 9.278i |
| $T_4$ | Control | 52.667c | 56.667b | 60.667a |
| LSD | | 2.3657 | | |

Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$).

**Table 5.** Evaluation of Levofloxacin and Ciprofloxin against citrus canker under field conditions.

| Sr # | Treatments | Disease incidence (%) |
|---|---|---|
| $T_1$ | Ciprofloxin | 24.09b |
| $T_2$ | Levofloxacin | 20.48c |
| $T_3$ | Levofloxacin + Ciprofloxin | 17.51d |
| $T_4$ | Control | 64.00a |
| | LSD | 1.4010 |

Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$).

In treatments and concentration interaction (T × C) Ciprofloxin expressed 27.22, 24.66 and 20.38, levofloxacin 24.22, 20.88, 16.33 percent disease incidence at 300, 500 and 700 ppm concentrations while minimum disease incidence was observed by the combination of both (Levofloxacin and Ciprofloxin) 21.22, 17.88 and 13.44 % respectively at three concentrations as compared to the control (Table 6 & Fig. 5).

**Table 6.** Impact of interaction between treatments and concentrations (T×C) against citrus canker under field conditions.

| Sr # | Treatments | Disease incidence (%) | | |
|---|---|---|---|---|
| | | Concentrations (%) | | |
| | | 1 | 2 | 3 |
| $T_1$ | Ciprofloxin | 27.22c | 24.66d | 20.38e |
| $T_2$ | Levofloxacin | 24.22d | 20.88e | 16.33f |
| $T_3$ | Levofloxacin + Ciprofloxin | 21.22e | 17.88f | 13.44g |
| $T_4$ | Control | 62.00b | 66.00a | 66.44a |
| | LSD | | 2.4266 | |

Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$).

**Table 7.** Impact of interaction between treatments and days (T×D) against citrus canker under field conditions.

| Sr # | Treatments | Disease incidence (%) | | |
|---|---|---|---|---|
| | | Days | | |
| | | After 10 days | After 20 days | After 30 days |
| $T_1$ | Ciprofloxin | 30.33d | 24.72ef | 17.22h |
| $T_2$ | Levofloxacin | 26.05e | 20.11g | 15.27h |
| $T_3$ | Levofloxacin + Ciprofloxin | 23.16f | 17.11h | 12.27i |
| $T_4$ | Control | 60.66c | 64.66b | 69.11a |
| | LSD | | 2.4266 | |

Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$).

Treatments and days interaction (T×D) exhibited that Ciprofloxin expressed 30.33, 24.72 and 17.22 % disease incidence while Levofloxacin (26.05, 20.11 and 15.27) and Levofloxacin + Ciprofloxacin 23.16, 17.11 and 12.27 % disease incidence when applied @ 1, 1.5 and 2 % after ten, twenty and thirty days respectively as compared to the control (Table 7 & Fig. 6).

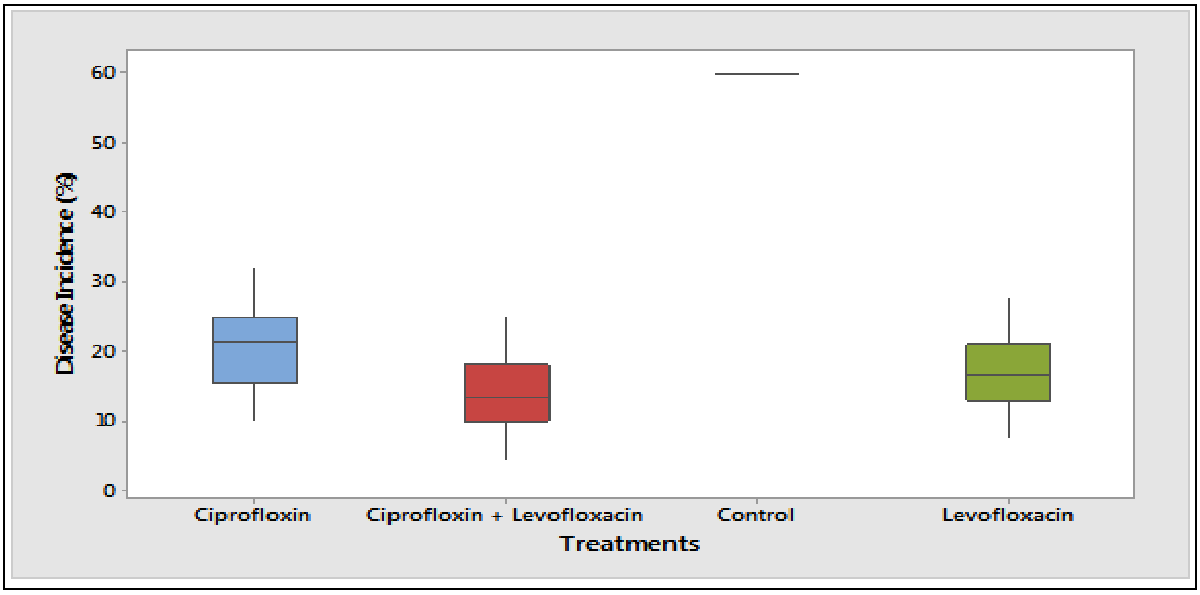


**Fig. 1.** Evaluation of treatments on the development of citrus canker under greenhouse conditions.

**Discussions**

Canker disease is a major issue for the citrus cultivation all over the world and causes huge losses every year. It caused by multiple strains of the *Xanthomonas axonopodis* pv. *citri* (Graham *et al,* 2004). No doubt, chemical management practices used for inhibiting the manipulation and multiplication of pathogen either by killing or blocking of metabolic pathways, but they cause severe hazardous effects on environment and human beings.

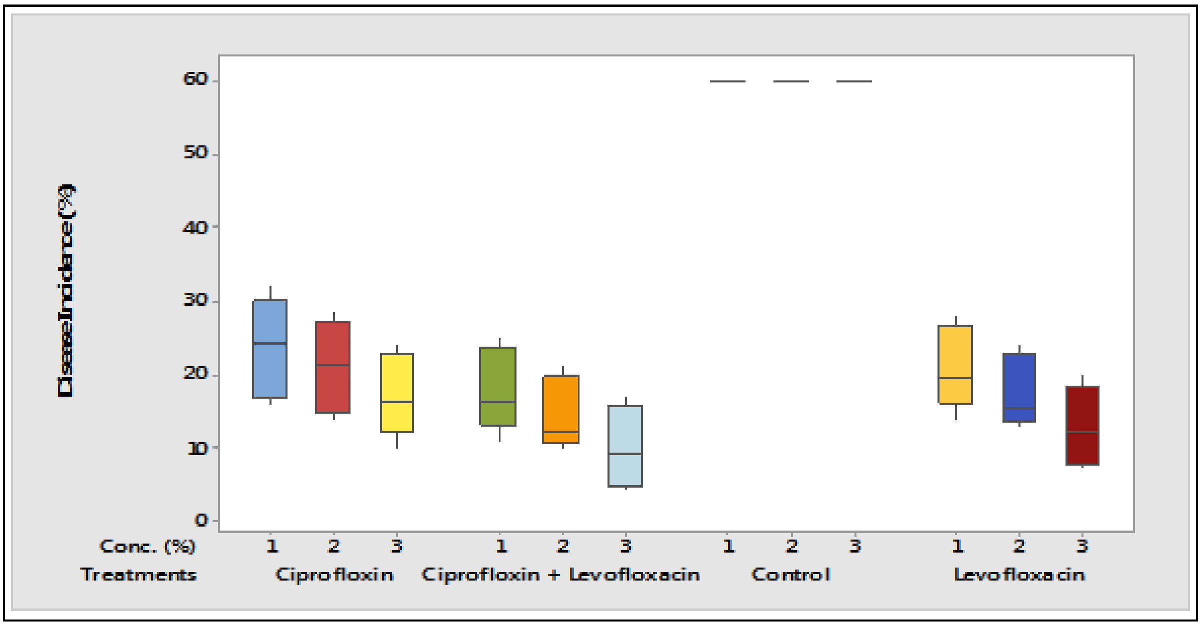


**Fig. 2.** Impact of interaction b/w treatments and concentrations (TxC) on the development of citrus canker under greenhouse conditions.

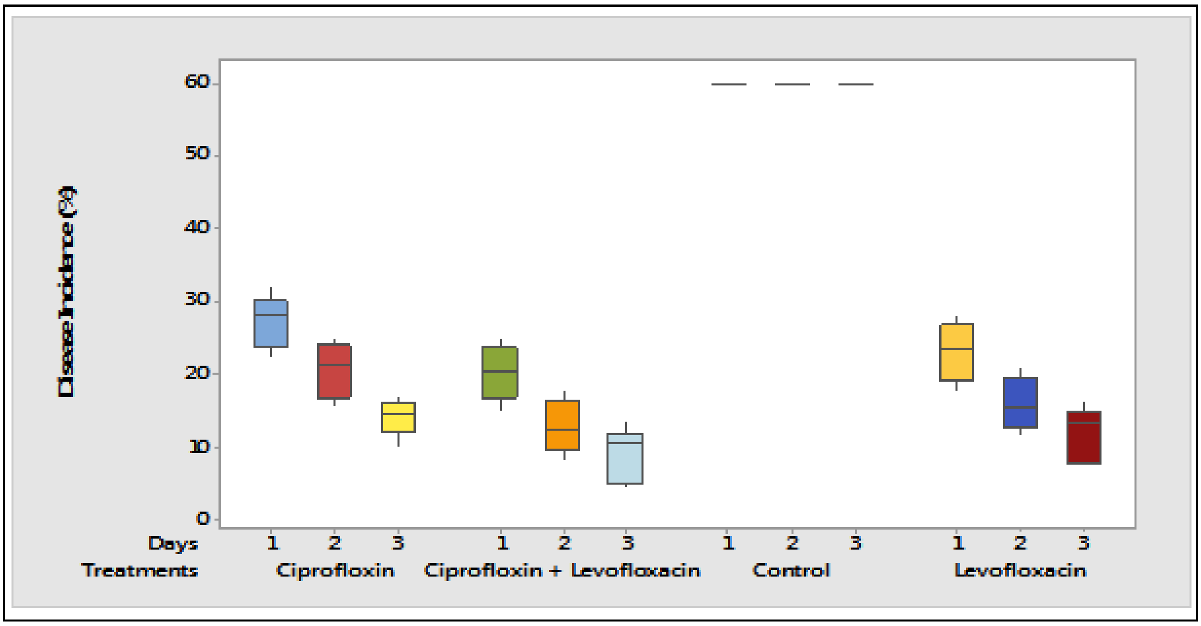


**Fig. 3.** Impact of interaction b/w treatments and days (TxD) on the development of citrus canker under greenhouse conditions.

The application of antibiotics is helpful in controlling plant diseases and in enhancing the resistance against the potential pathogens (Islam *et al.,* 2014). Development of drug-resistant strains causes various serious problems' formulating fool-proof control (Gnanamanickam *et al,* 1999). Fitt *et al,* (1992) recorded that few antibiotics can be used for controlling bacterial growth; according to their conclusion penicillin/dihydro-streptomycin and chloramphenicol were proved most effective during first 48 h. Erasmus *et al*, (1997) also observed that cefotaxime and chloramphenicol at 150 mg and 20 mg mL–1 respectively inhibited and suppressed the bacterial growth.

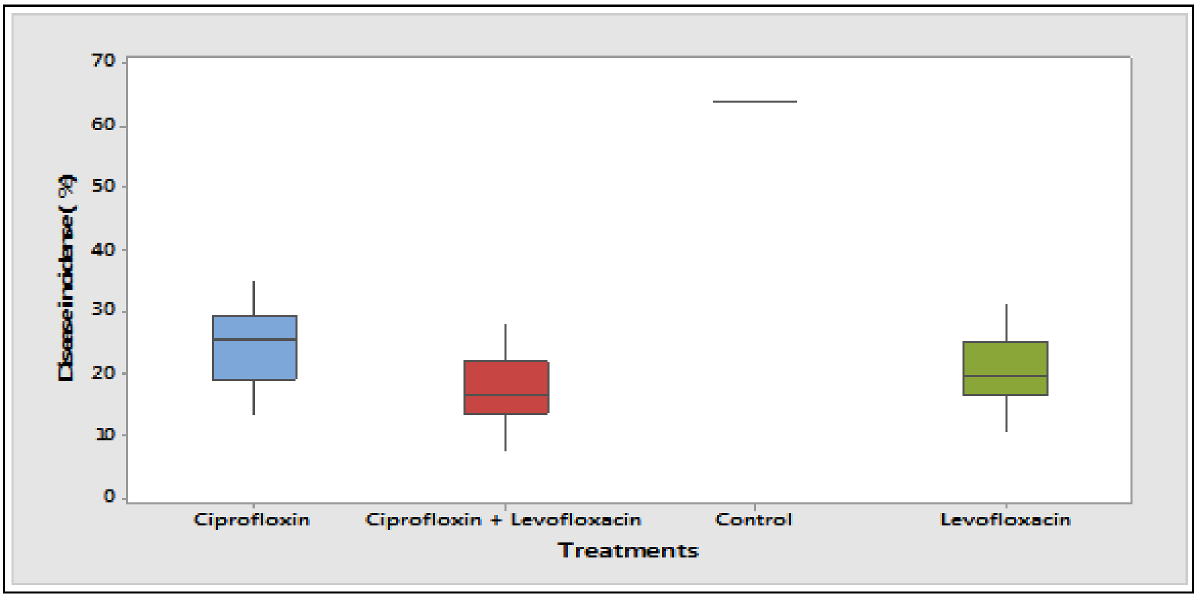


**Fig. 4.** Evaluation of antibiotics on the development of citrus canker under field conditions.

In the present studies, efficacy of various antibiotics was checked for management of the disease. A total 9 antibiotics were tested against *Xanthomonas axonopodis* pv. *citri* under *in-vitro* conditions. Levofloxacin at 700 ppm concentration was recorded best against *Xac*. Outcomes of present study was supported by the findings of Christiano *et al*, 2010 who studied the effect of different antibiotics for controlling bacterial spots of stone fruits (peach and nectarine) and concluded that Oxytetracycline expressed the best result against the *Xanthomonas arboricola* pv. *pruni*.

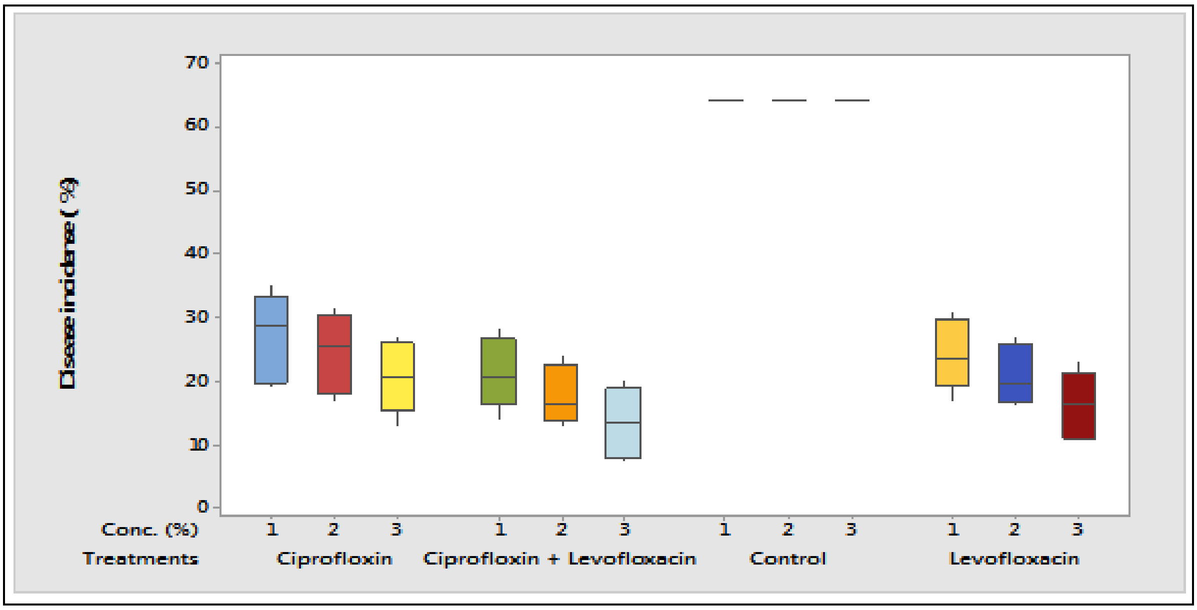


**Fig. 5.** Impact of interaction b/w treatments and concentrations (TxC) on the development of citrus canker under field conditions.

It acts as a bacteriostatic and prevent the multiplication of bacteria by binding with chromosomes. Oxalinic acid is found best in Japan and registered for the management of bacterial panicle blight of rice, caused by *Burkhulderia glumaea* (Nandakumar *et al*, 2010).

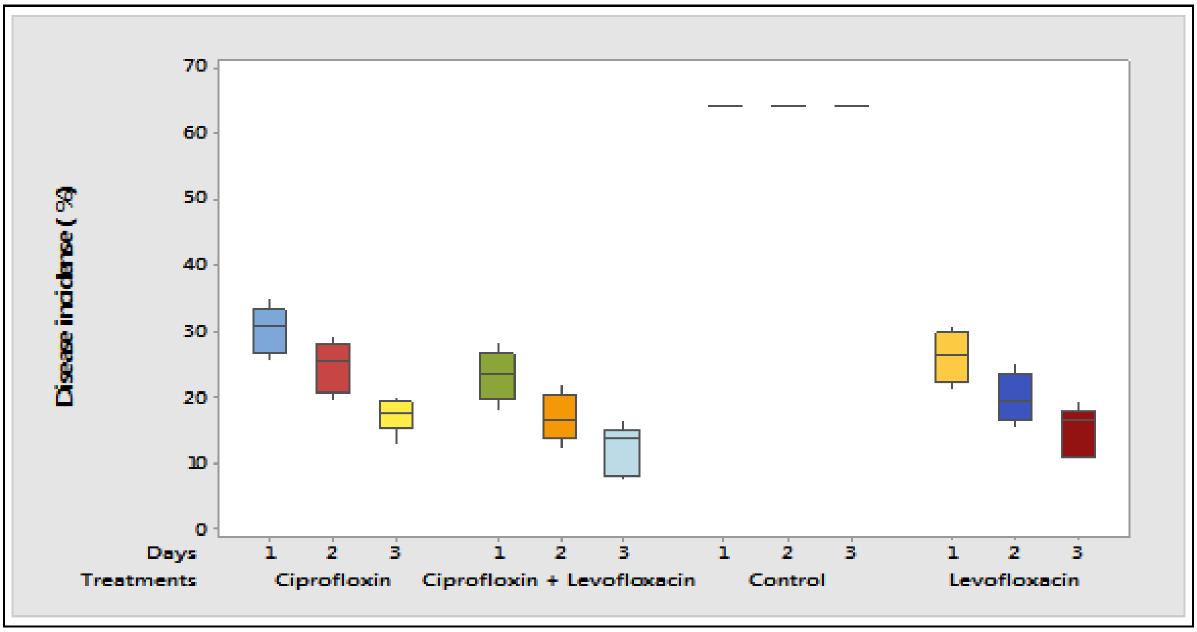


**Fig. 6.** Impact of interaction b/w treatments and days on the development of citrus canker under field conditions.

They revealed that oxalinic acid stopped the DNA replication and ultimately bacterial growth by inactivating the target DNA enzymes like gyrazeand topoisomerase IV. Francis *et al*, 2010 who observed that high levels of penicillin sodium/ dihydrostreptomycin and chloramphenicol decreases bacterial population.

Jones A.L. & Schnabel E.L, (2000) also studied the effect of streptomycin on the fire blight of apple and pear caused by *Erwinia amylovora* and concluded that these antibiotics are bactericidal, irreversibly bind with the bacterial chromosomes, cause a spontaneous mutation in the rpsl chromosomal gene which is directly responsible for the synthesis of proteins.

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**Conflict of interest statement**

The author (s) declares (s) that there is no conflict of interest.

**References**

**Christiano RSC, Reilly CC, Miller WP, Scherm H.** 2010. Oxytetracycline dynamics on peach leaves in relation to temperature, sunlight, and simulated rain. Plant Disease **94,** 1213-1218.

**Deng ZN, Xu L, Li DZ, Long GY, Liu LP, Fang F, Shu GP.** 2009. Screening citrus genotypes for resistance to canker disease (*Xanthomonas axonopodis* pv. *citri*). Plant Breeding **129,** 341-345.

**Erasmus JH, Cook PA, Coyne VE.** 1997. The role of bacteria in the digestion of seaweed by the abalone H. midae. Aquaculture **155,** 377-386.

**Fitt WK, Heslinga GH, Watson TC.** 1992. Use of antibiotics in the maricultural of giant clams (*F. Tridacnidae*). Aquaculture **104,** 1-10.

**Francis MI, Peña A, Graham JH.** 2010. Detached leaf inoculation of germplasm for rapid screening of resistance to citrus canker and citrus bacterial spot. European journal of plant pathology **127(4),** 571-578.

**Gnanamanickam SS, Priyadarisini VB, Narayanan NN, Vasudevan P, Kavitha S.** 1999. An overview of bacterial blight disease of rice and strategies for its management. Current Science **77(11),** 1435-1444.

**Gottwald TR, Sun X, Riley T, Graham JH, Ferrandino F, Taylor EL.** 2002. Geo-Referenced Spatiotemporal Analysis of the Urban Citrus Canker Epidemic in Florida. Phytopathology **92,** 361-377.

**Graham JH, Gottwald TR, Cubero J, Achor DS.** 2004. *Xanthomonas axonopodis* pv. *citri*: factors affecting successful eradication of citrus canker. Molecular plant pathology **5(1),** 1-15.

**Heinlaan M, Ivask A, Blinova I, Dubourguier HC, Kahru A.** 2008. Toxicity of nano sized and bulk ZnO, CuO and TiO2 to bacteria *Vibrio fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus*. Chemosphere **71,** 1308-1316.

**Hemraj V, Diksha S, Avneet G.** 2013. A review on commonly used biochemical test for bacteria. Innovare Journal of Life Sciences **1,** 1-7.

**Husak VV.** 2015. Copper and copper-containing pesticides: metabolism, toxicity an oxidative stress. Journal of Vasyl Stefanyk Precarpathian National University **1,** 39-51.

**Islam MA, Mazumdar RM, Islam S, Alam MJ, Urmee SA.** 2014. Isolation, identification and *in-vitro* antibiotic sensitivity pattern of citrus canker causing organism *Xanthomonas axonopodis*. Advances in life sciences **1(4),** 215-222.

**Jones AL, Schnabel EL.** 2000. The development of streptomycin-resistant strains of *Erwinia amylovora*. In Fire blight: the disease and its causative agent, *Erwinia amylovora* (J. Vanneste, ed.). CAB International, Wallingford UK, 235–251.

**Memon NA.** 2017. Citrus fruit (kino): Punjab produced 98% of production. Pakistan Bureau of statistics 2016-2017.

**Mostafalou S, Abdollahi M.** 2013. Pesticides and human chronic diseases: evidences, mechanisms, and perspectives. Toxicology and applied pharmacology **268,** 157-177.

**Mubeen M, Arshad H, Iftikhar Y, IrfanUllah M, Bilqees I.** 2015. Bio-chemical characterization of *Xanthomonas axonopodis* pv. *citri*: a gram-negative bacterium causing citrus canker. International Journal of Science and Nature **6,** 151-154.

**Nandakumar R, Shahjahan AKM, Yuan XL, Dickstein ER, Groth DE, Clark AC, Cartwright RD, Rush MC.** 2009. *Burkholderia glumae* and *B. gladioli* cause bacterial panicle blight in rice in the southern United States. Plant Disease **93,** 896–905.

**Rehman A, Imran M, Mehboob S, Nasir AK, Riaz K.** 2015. Etiology Pathogenicity and Management of Collar Rot in Cockscomb (*Celosia argentea).* International Journal of Agriculture and Biology **17(1).**

**Riaz K, Elmerich C, Raffoux A, Moreira D, Dessaux Y, Faure D.** 2008. Metagenomics revealed a quorum quenching lactonase QlcA from yet unculturable soil bacteria. Communications in agricultural and applied biological sciences **73,** 3-6.

**Ruangpan L, Tendencia EA.** 2004. Laboratory manual of standardized methods for antimicrobial sensitivity tests for bacteria isolated from aquatic animals and environment. Aquaculture Department, Southeast Asian Fisheries Development Center.

**Wali S, Munir F, Mahmood T.** 2013. Phylogenetic studies of selected Citrus species based on chloroplast gene, rps14. International Journal of Agriculture and

Biology **15,** 357-361.

**Ware M.** 2015**.** Oranges: Health benefits, nutritional information.

**Zhang W, Jiang F, Ou J.** 2011. Global pesticide consumption and pollution: with China as a focus. Proceedings of the International Academy of Ecology and Environmental Sciences **1,** 125.