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In-vivo and *in-vitro* management of citrus canker disease through potential and cheap antibiotics

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Abstract

Current study was conducted on *in-vivo* and *in-vitro* management of citrus canker disease through potential and cheap antibiotics. Different antibiotics were assessed against canker disease. Under *in-vivo* experiment trees were randomly selected and sprayed with antibiotics. Under *in-vitro* lemon fruits were treated with different antibiotics. The data was recorded on a daily basis for the number and size of lesions developed on leaves, branches, and fruits. *In-vivo* effect of different antibiotics showed that streptomycin had highest efficacy followed by pencilin-10, Penicilin-5 and zampicilin for leaves, branches, and fruits. 15 days after application, while in case of 45 days after 1st spray and 15 days after 2nd spray, the same was increased in Streptomycin, Pencilin-10, Pencilin-5 and zampicilin for leaves, branches, and fruits. However, the effect of different antibiotics based on tree directions, results were non-significant. On overall, streptomycin found to be the best followed by pencilin-10, Pencilin-5, and zampciline. The *in-vitro* efficacy of streptomycin was most effective on un-injured and injured fruits, followed by Pencillin-10, pencillin-5 on injured and un-injured; while the lowest efficacy was recorded in case of zampicillin and there was no infection in Control (even Injured or un-injured un inoculated and un-treated). Significant reduction in the size of lesion was obtained with streptomycin when applied in injured and un-injured followed by Pencillin-5 in un-injured, Pencillin-10 in un-injured; while Pencillin-5 in injured followed by pencillin-10 and Zampicillin injured found low effective antibiotic followed by zampicilin un-injured. There was no disease in injured and un-injured un-inoculated control; while the minimum disease percent was observed in streptomycin un-injured, followed by streptomycin injured and pencilin-10 un-injured, pencilin-10 injured, pencillin-5 un-injured, Pencillin-5 injured and Zampicilin un-injured and injured. The results so far achieved in the current study are hoped will be helpful for the grower in sustainable management of citrus canker disease.

Keywords: Cheap antibiotics, Citrus canker, *In-vitro, In-vivo*, Management

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Introduction

Citrus is a collective generic term, comprising several species and varieties of fruits, popular at global level for their characteristics flavor and attractive range of colors. Its attractive evergreen foliage and fragrance are added aesthetic value of citrus trees as well (Meena et al., 2018). Citrus is known by people since vestige and is a decent source of nutrient "C" with high cancer prevention agent potential (Gorinstein et al., 2001). Among the citrus fruits, lemon, Citrus limon L. Burm. F., is one of the most important members of the large Rutaceae family, including about 130 genera in seven subfamilies that are commonly used for several purposes worldwide (Lucker et al., 2002; AL-Jabri and Hossain, 2014).

Though the origin of lemons is uncertain; however, it grows well under conducive climate of both tropical and semi tropical regions of the world and the major proportion of production is mainly limited to sub-tropical regions. The bright attractive yellow color, and excessively high acid content are the main characteristics of lemon; they are mainly used to prepare the juice, lemonade, as well as lemon oil and pectin (Kimbal, 1999). It contains ascorbic acid, commonly called as vitamin C, which is lower than oranges and grapes but higher than in lime fruits at varying storage temperatures (Njoku et al., 2011).

Many biotic factors effect profitable production of citrus (Kiptoo et al., 2021; Umar et al., 2021; Iqbal et al., 2022; Rehman et al., 2022). Among all these, citrus canker caused by the bacterium Xanthomonas citri subsp. citri (also known as *X*. axonopodis pv. citri, and *X*. campestris pv. citri) is believed to be a severe threat to citrus (Gottwald, 2000; Gottwald et al., 2002). The wet tropical and subtropical citrus growing areas of the world are very conducive, where the disease occurs due the splash of rain (Bock et al., 2005; Bock et al., 2006; Pruvost et al., 2002). It mainly encounters the plant through natural opening or wounds special on tender leaves, stems, thorns, and fruit causing necrotic lesion or canker at the infection site, defoliation of leaves, badly blemished fruit, premature fruit drop, twig dieback and overall tree decline. The disease deteriorating quality and quantity of fruits, which are unsuitable for the fresh, market, thus, resulting huge economic loss (Civerolo, 1984; Schubert et al., 2001).

Citrus canker may result in large economic damages to the citrus production worldwide each in terms of loss to trees (mostly reduced fruit production), reduced access to trade markets, or the costs of its avoidance and control. Spots shows on leaves, twigs and fruits which cause defoliation, and can finally kill the tree. It is easily disseminated in different areas through diseased plant debris, infected fruits and seedlings, and careless re-introduction is highly possible despite the quarantine limits that are in place in several countries. Nearby, X. citri is quickly disseminated by rainwater running over the surfaces of lesions and move on uninfected shoots; it is also spread under conditions of high temperature, heavy rainfall and solid winds. Certain areas of the world have eliminated citrus canker, others have on-going eradication programme; however, this bacterium remains a danger to all citrus-growing areas (CABI, 2018).

Citrus canker is managed with protective sprays of copper-based bactericides throughout the worldwide (Hartung, 1992; Leite, 1990; Leite et al., 1987; Stall and Seymour, 1983; Kuhara, 1978). Such bactericides are used to decrease inoculum build up on tender leaves and to protect increasing fruit surfaces from infection. Actual destruction of the disease by copper sprays depends on numerous aspects, such as the weakness of the citrus cultivar, environmental conditions, and using of other management practices (Leite and Mohan, 1990; Hartung, 1992; Leite, 1990; Leite et al., 1987; Stall and Seymour, 1983; Kuhara, 1978). As a stand-alone measure, control of citrus canker with copper sprays on resistant or moderately resistant citrus cultivars may be achieved, whereas adequate control on susceptible or highly susceptible cultivars requires the implementation of several control measures (Leite et al., 1987; Leite and Mohan, 1984; Stall and Seymour, 1983; Kuhara, 1978). A field trials conducted in Brazil demonstrate that even reduced rates of copper formulations (copper hydroxide, CH; copper oxychloride, COC) are consistently effective for control of canker (*X.* axonopodis pv. citri) on moderately susceptible orange varieties (Graham 2006).

In addition, the timing and number of bactericides sprays for effective control of citrus canker is not only highly dependent on the susceptibility of the citrus cultivar, but on the age of the citrus trees, environmental conditions, furthermore, the selection of other control measures. In general, 3 to 5 sprays are necessary for effective control of citrus canker on citrus cultivars with in-between levels of resistance (Leite, 1990), whereas, in years with weather that is highly favorable for epidemic development of citrus canker, up to 6 sprays may be suggested (Leite et al., 1987).

Materials and Methods

The experiment and studies on *in-vivo* and *in-vitro* management of citrus canker disease through potential and cheap antibiotics was conducted in the Department of Plant Pathology, Faculty of Crop Protection, Sindh Agriculture University Tandojam, Pakistan during 2017 to 2019. The materials and methods carried out as per plan of work are described below.

In-vitro and *in-vivo* management of citrus canker disease of lemon

Different available antibiotics were assessed *in-vitro* and *in-vivo* against canker disease to know the potential and cheap antibiotics. Lemon fruits (intentionally injured and un-injured) were inoculated with canker causing pathogen and then was treated with different antibiotics under *in-vitro*. Un-inoculated and untreated lemon fruits were kept as control. The experiment was conducted in complete randomized design (CRD) with eight replications. All the treatments were incubated at room temperature. Under *in-vivo* experiment trees were randomly selected and sprayed with antibiotics. Data was recorded on a daily basis for the number and size of lesions developed on fruits and leaves.

Statistical analysis

The recorded data was analysed by using “Statistix 8.1” a statistical package of computer software.

Results

In-vivo effect of different cheap antibiotics on CCD

Effect of different cheap antibiotics on percent of infected leaves of lemon

The effect of different antibiotics on the percentage of infected leaves represents that different antibiotics were found effective against the disease. Streptomycin was found to be the best followed by Pencilin-10, Pencilin-5 and Zampicilin as compare to control. Streptomycin showed (56.24%) followed by Pencilin-10 (45.83%), Penicilin5 (39.58%) and Zampicilin (27.08%) 15 days after application, while in case of 45 days after 1st spray and 15 days after 2nd spray, the same was increased in Streptomycin (64.51%), Pencilin10 (54.84%), Penicilin5 (45.16%) and Zampicilin (33.87%) (Table 1).

The effect of different antibiotics on the basis of tree directions, the results was non-significant. However, 35.00%) efficacy was recorded on the west and north side of the trees, while in south it was (33.33%) and in east, the same was (31.66%), 15 days after 1st spray. It was increased to on the 45 days after 1st (and 15 days after 2nd) spray as (41.29%) on south, (40.00%), (40.00%) and 37.420% on east, west and north side of the trees (Table 2).

Table 1 *In-vivo* effect of different cheap antibiotics on percent of infected leaves of lemon trees

Treatments	Before 1st spray	15 days after 1st spray	Net infected leaves after 15 days after 1st spray	Efficacy (%)
Zampicillin	4.50 a	8.75 b	4.25 b	27.082 c
Pencillin-10	4.25 a	6.50 cd	2.25 bc	45.835 b
Pencillin-5	5.50 a	7.25 c	1.75 bc	39.582 b
Streptomycin	4.50 a	5.25 d	0.75 c	56.248 a
Control	4.75 a	12.75 a	8.00 a	0.000 d
Significant	N.S.	H.S.	H.S.	H.S.
S.E.	0.9287	0.6124	1.2349	3.6874
C.V @ 0.05	2.0235	1.3342	2.6906	8.0342
Treatments	Before 2nd spray 30 days after first	15 days after 2nd spray (45 days after first)	Infected leaves 15 days after 2nd spray (45 days after first spray)	Efficacy (%)
Zampicillin	5.25 b	10.25 b	5.00 a	33.870 d
Pencillin-10	5.00 b	7.00 d	2.00 b	54.840 b
Pencillin-5	6.75 b	8.50 c	1.75 bc	45.165 c
Streptomycin	5.50 b	5.50 e	0.00 c	64.515 a
Control	11.25 a	15.50 a	4.25 bc	0.0000 e
Significant	N.S.	H.S.	H.S.	H.S.
S.E.	0.8732	0.6455	0.8921	3.6779
C.V @ 0.05	1.9026	1.4064	1.9437	8.0136

Table 2 *In-vivo* effect of different cheap antibiotics on percent of infected leaves of lemon at different directions of tree

Directions	Before 1st spray	15 days after 1st spray	Infected leaves after 15 days after 1st spray	Efficacy (%)
East	4.60 ab	8.40 a	3.80 a	31.666 a
West	5.60 a	8.20 a	2.60 a	35.000 a
North	3.40 b	8.00 a	4.60 a	35.000 a
South	5.20 ab	7.80 a	2.60 a	33.332 a
Significant	N.S.	N.S.	N.S.	N.S.
S.E.	0.8307	0.5477	1.1045	3.2981
C.V@0.05	1.8099	1.1934	2.4066	7.1860
Directions	Before 2nd spray 30 days after first	15 days after 2nd spray (45 days after first)	Infected leaves 15 days after 2nd spray (45 days after first spray)	Efficacy (%)
East	6.60 a	9.40 a	2.80 ab	40.002 a
West	7.20 a	9.20 a	2.00 ab	40.00 a
North	6.20 a	10.00 a	3.800 a	37.420 a
South	7.00 a	7.00 a	1.800 b	41.290 a
Significant	N.S.	N.S.	N.S.	N.S.
S.E.	0.7810	0.5774	0.7979	3.2897
C.V@0.05	1.7017	1.2579	1.7385	1.1675

Effect of different cheap antibiotics on percent of infected branches of lemon

The effect of different antibiotics on the percentage of infected branches represents different antibiotics found effective against the disease. Streptomycin was found to be the best followed by Pencillin-10, Penicillin-5 and Zampicilin as compared to control. Streptomycin showed (63.33%) followed by Pencilin-10 (40.00%), Penicilin-5 (26.66%) and Zampicilin (13.35%) 15 days after application, while in case of 45 days after 1st spray and 15 days after 2nd spray, the same was increased in Streptomycin (70.59%), Pencilin-10 (55.88%), Penicilin-5 (29.41%) and Zampicilin (11.76%) (Table 3).

The effect of different antibiotics based on tree directions, the results was non-significant. However, (31.99%) efficacies were recorded on the west side of the trees, while in north it was (29.33%), east, (26.68%) and in (26.66%) south, the same was (31.66%) (Table 4). It was increased to on the 45 days after 1st (and 15 days after 2nd) spray as (37.65%) on south and west, (30.55%) east and (28.23%) on north, side of the trees.

Table 3 *In-vivo* effect of different cheap antibiotics on percent of infected branches of lemon trees

Treatments	Before 1st spray	15 days after 1st spray	Infected leaves after 15 days after 1st spray	Efficacy (%)
Zampicillin	1.75 b	6.50 b	4.75 a	13.350 d
Pencillin-10	1.25 b	4.50 d	3.25 b	40.000 b
Pencillin-5	3.00 a	5.50 c	2.50 b	26.665 c
Streptomycin	2.50 a	2.75 e	0.25 c	63.332 a
Control	2.75 a	7.50 a	4.75 a	0.0000 e
Significant	N.S.	H.S.	H.S.	H.S.
S.E.	0.2500	0.4183	0.4743	4.8687
C.V @ 0.05	0.5447	0.9115	1.0335	10.608
Treatments	Before 2nd spray 30 days after first	15 days after 2nd spray (45 days after first)	Infected leaves 15 days after 2nd spray (45 days after first spray)	Efficacy (%)
Zampicillin	2.75 b	7.50 a	4.75 a	11.765 d
Pencillin-10	3.50 c	3.75 c	0.25 c	29.415 c
Pencillin-5	4.00 b	6.00 b	2.00 b	55.885 b
Streptomycin	2.50 c	2.50 d	0.000	70.590 a
Control	5.00 a	8.50 a	3.500 a	0.0000 d
Significant	N.S.	H.S.	H.S.	H.S.
S.E.	0.6258	0.4830	0.6551	5.9792
C.V @ 0.05	1.3636	1.0525	1.4274	13.027

Table 4 *In-vivo* effect of different cheap antibiotics on percent of infected branches of lemon at different direction of trees

Directions	Before 1st spray	15 days after 1st spray	Infected leaves after 15 days after 1st spray	Efficacy (%)
East	2.40 b	5.40 a	3.00 b	26.668 a
West	1.80 c	5.20 a	3.40 ab	31.998 a
North	3.20 a	5.20 a	2.00 c	29.334 a
South	1.60 c	5.60 a	4.00 a	26.666 a
Significant	H.S.	N.S.	H.S.	N.S.
S.E	0.2236	0.3742	0.4243	4.3547
C.V@0.05	0.4872	0.8152	0.9244	9.4880
Directions	Before 2nd spray 30 days after first	15 days after 2nd spray (45 days after first)	Infected leaves 15 days after 2nd spray (45 days after first spray)	Efficacy (%)
East	3.40 a	6.00 ab	2.60 a	30.588 a
West	3.40 a	5.20 b	1.80 a	37.650 a
North	4.00 a	6.20 a	2.20 a	28.236 a
South	3.40 a	5.20 b	1.80 a	37.650 a
Significant	N.S.	N.S.	N.S.	N.S.
S.E	0.5598	0.4320	0.5859	5.3479
C.V@0.05	1.2196	0.9414	1.2767	11.652

Effect of different cheap antibiotics on percent of infected fruits of lemon

The effect of different antibiotics on the percentage of infected fruits represents those different antibiotics found effective against the disease. Streptomycin was found to be the best followed by Pencillin-10, Pencillin-5 and Zampicilin as compared to control. Streptomycin showed (69.99%) followed by Pencillin-10 (5.00%), Penicilin-5 (33.33%) and Zampicilin (13.33%) 15 days after 1st spray, while in case of 45 days after 1st spray and 15 days after 2nd spray, the same was increased in Streptomycin (63.33%), Pencilin-10, (46.66%), Penicilin-5, (26.66%) and Zampicilin (16.66%) (Table 5). The effect of different antibiotics based on tree directions; the results was non-significant. However, (37.33%) efficacy was recorded on the north side of the trees, while in south, east and west it was (32.00%) it was increased to on the 45 days after 1st (and 15 days after 2nd) spray as (32.00%) on south and west, while the east (29.33%), and north (29.33%) side of the trees (Table 6).

Table 5 *In-vivo* effect of different cheap antibiotics on percent of infected fruits of lemon trees

Treatments	Before 1st spray	15 days after 1st spray	Infected leaves after 15 days after 1st spray	Efficacy (%)
Zampicillin	2.25 bc	6.50 a	4.25 a	13.335 d
Pencillin-10	2.75 ab	3.75 c	1.00 b	5..003 b
Pencillin-5	3.50 a	5.00 b	1.50 b	33.333 c
Streptomycin	1.75 c	2.25 d	0.50 b	69.997 a
Control	3.00 b	7.50 a	4.50 a	0.0000 e
Significant	N.S.	H.S.	N.S.	H.S.
S.E	0.4233	0.4610	0.7416	5.3742
C.V @ 0.05	0.9222	1.0044	1.6159	11.709
Treatments	Before 2nd spray 30 days after first	15 days after 2nd spray (45 days after first)	Infected leaves 15 days after 2nd spray (45 days after first spray)	Efficacy (%)
Zampicillin	3.25 bc	6.25 b	3.00 a	16.668 c
Pencillin-10	4.00 ab	4.00 c	0.00 b	46.668 b
Pencillin-5	4.00 ab	5.50 b	1.50 ab	26.665 c
Streptomycin	2.50 c	2.75 d	0.25 b	63.332 a
Control	4.50 a	7.50 a	3.00 a	0.0000 d
Significant	N.S.	H.S.	N.S.	H.S.
S.E	0.5083	0.4610	0.7416	6.8303
C.V @ 0.05	1.1074	1.0044	1.6159	14.882

Table 6 *In-vivo* effect of different cheap antibiotics on percent of infected fruits of lemon at different direction of trees

Directions	Before 1st spray	15 days after 1st spray	Infected leaves after 15 days after 1st spray	Efficacy (%)
East	3.400 a	5.00 a	1.600 a	32.00 a
West	2.200 a	5.20 a	3.00 a	32.00 a
North	2.80 ab	4.800 a	2.00 a	37.334 a
South	2.200 b	5.00 a	2.80 a	32.00 a
Significant	N.S.	N.S.	N.S.	N.S.
S.E	0.3786	0.4123	0.6633	4.8068
C.V@0.05	0.8249	0.8983	1.4453	10.473
Directions	Before 2nd spray 30 days after first	15 days after 2nd spray (45 days after first)	Infected leaves 15 days after 2nd spray (45 days after first spray)	Efficacy (%)
East	4.00 a	5.40 a	1.40 a	29.334 a
West	3.40 a	5.00 a	1.60 a	32.000 a
North	4.00 a	5.40 a	1.40 a	29.332 a
South	3.20 a	5.00 a	1.80 a	32.000 a
Significant	N.S.	N.S.	N.S.	N.S.
S.E.	0.4546	0.4726	0.6633	6.1092
C.V@0.05	0.9905	1.0297	1.4453	13.311

Effect of different antibiotics on number of citrus canker lesions/fruit of lemon

The effect of different antibiotics on the number of canker lesions/fruit shows 5 groups in which means are not significantly different from one another. The minimum number of lesions were counted in Streptomycin (0.43) and Penicillin-10 (1.18) followed by Penicillin-5 (1.43) and Zampicillin (1.56) as compared to control (3.87) (Fig. 1) whereas, there were no disease symptoms in un-inoculated treated. The SE and CV for comparison were 0.1543 and 0.3073, respectively. On an overall, there are 2 groups in which the means are highly significantly different from one another at alpha 0.05 and statically the result enumerates maximum lesions in Injured (1.58) followed by un-injured (1.25); where SE for comparison was 0.0891 and the CV for comparison was 0.1774. The data with reference to interaction between antibiotic and inoculation method represents 7 groups in which the means are not significantly different from one another at alpha 0.05 and that the minimum number of lesions were recorded in un-injured and injured fruits treated with Streptomycin (0.25 and 0.62), followed by un-injured fruits treated with Penicillin-10 (1.12), injured fruits treated with Penicillin 10 and un-injured fruits treated with penicillin 5 (1.25), un-injured fruits treated with Zampicillin (1.37), injured fruits treated with Penicillin-5 (1.62) and with Zampicillin (1.75) as compare to control (3.50 uninjured and 4.25 injured). There were no canker lesions in injured and un-injured control (Fig. 1). The SE and CV for comparison were 0.2182 and 0.4345 at alpha 0.05.

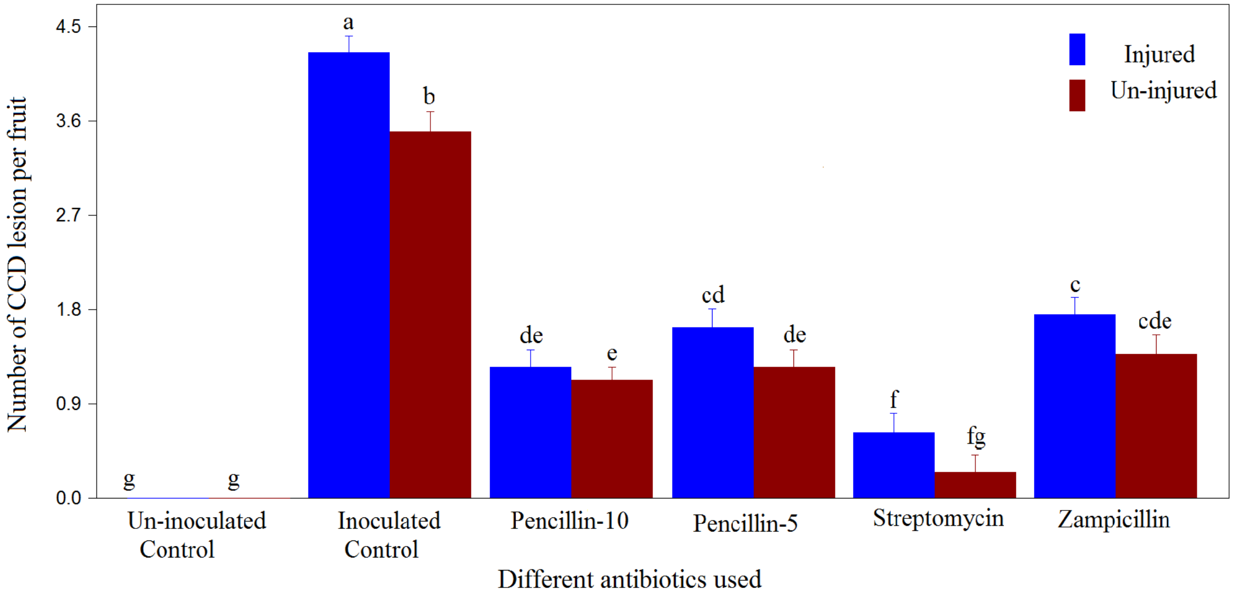

Fig. 1 Effect of different antibiotics on number of citrus canker lesions /fruit of lemon

Effect of different antibiotics on length of citrus canker lesions on fruit of lemon

The effect of different antibiotics on length of canker lesions on fruit demonstrated 4 groups in which the means are not significantly different from one another. There was no spot in un-inoculated control, hence, no length (0.00) was recorded but the lowest spots length was noted in Streptomycin (0.05) followed by Penicillin-5 (0.55), Penicillin-10 (0.67) and Zampicillin (0.67) (Fig. 2). Significantly the highest spot length was recorded in inoculated control (1.10). Where SE for comparison was 0.0438 and the CV for comparison was 0.0873 at alpha 0.05. The overall mean of inoculation methods shows 2 groups in which the means were highly significantly different from one another at alpha 0.05. The highest spots length was observed in injured (0.64) as compared to un-injured fruits (0.37); where SE and the CV for comparison was 0.0253 and 0.0504. The interaction between treatment and methods represents 7 groups in which the means are not significantly different from one another at alpha 0.05. The lowest length was recorded in Streptomycin applied in un-injured (0.02) followed by Streptomycin in injured (0.07), Pencilin-5 un-injured (0.17), Pencilin-10 un-injured (0.44), Zampicillin un-injured (0.56), Zampicillin injured (0.78), Pencilin-10 injured (0.90) and Penicillin-5 injured (0.92); while the highest number of spots were recorded in inoculated control injured and un-injured (1.20 and 1.01), but no spots were appeared in the un-inoculated control (Fig. 2). The SE and CV for comparison were 0.0620 and 0.1234, respectively.

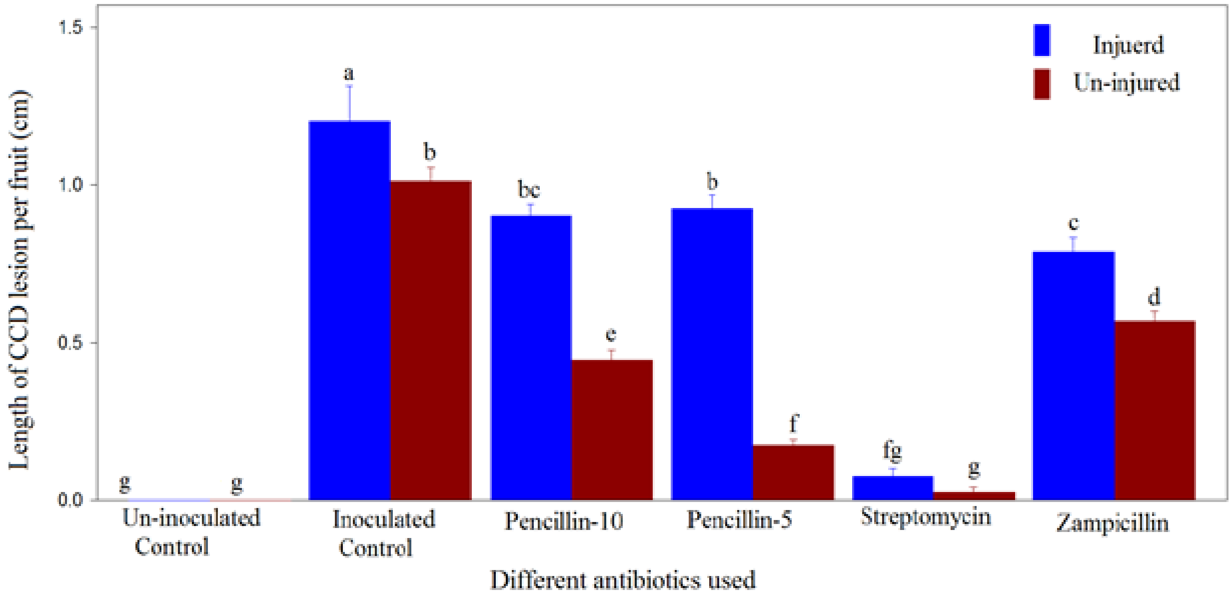

Fig. 2 Effect of different antibiotics on length of citrus canker lesions /fruit of lemon

Effect of different antibiotics on the width of citrus canker lesions on fruit of lemon

The effect of different antibiotics on the width of canker lesions on fruit showed 4 groups in which the means are not significantly different from one another. There was no spot hence no width in un-inoculated control (0.00) was recorded but the lowest spot width was found in Streptomycin (0.04), followed by Penicillin-5 and 10 (0.5781 and 0.6094), while the highest spot width recorded in inoculated control (1.08) followed by and Zampicillin (0.67). Where the SE and the CV for comparison was 0.0650 and 0.0326 at alpha 0.05. However, there are 2 groups in which the means were highly significantly different from one another at alpha 0.05 with reference to overall mean and that the lengthiest spots width found in injured fruits (0.63) as compare to un-injured fruits (0.35). Where SE and the CV for comparison was 0.0188 and 0.0375. The interaction between treatment and method showed 7 groups in which the means are not significantly different from one another with 0.462 and 0.0919 SE and CV for comparison at alpha 0.05. There was no appearance of spots in un-inoculated control injured and un-injured (0.00), the lowest spots width in the Streptomycin applied on un-injured and injured fruits (0.02 and 0.62) followed by Penicillin-5 un-injured (0.22), Pencilin-10 un-injured (0.41), Zampicillin un-injured (0.54), Pencilin-10 injured and Zampicillin injured (0.80) and 0.8063 (Fig. 3). Significantly maximum spot width was recorded in inoculated injured control (1.23) followed by Penicillin-5 injured (0.93) and inoculated un-injured control (0.9225) (Fig. 3).

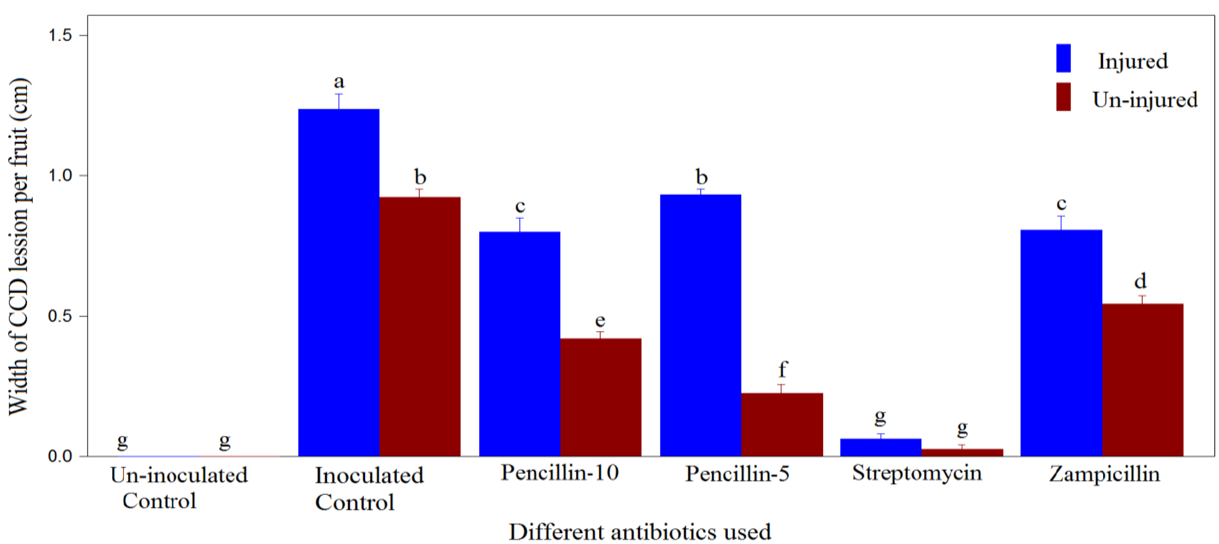

Fig. 3 Effect of different antibiotics on the width of citrus canker lesions (cm) on fruits of lemon

Effect of different antibiotics on the total size of citrus canker lesions on fruit of lemon

Effect of different antibiotics on total canker lesion size (length + width) illustrated 4 groups in which the means are not significantly different from one another. Significantly, there was no spot hence no lesion size in un-inoculated control (0.00), but was lowest in Streptomycin (0.09), followed by Penicillin-5 (1.12). The highest spots size was recorded in inoculated control (2.18) followed by Zampicillin and Penicillin-10 (1.28 and 1.35), respectively. Whereas SE and the CV for comparison was 0.0643 and 0.1280 at alpha 0.05, respectively. On an overall mean basis, there are 2 groups in which the means were highly significantly different from one another at alpha 0.05 with the highest size in injured fruits (1.28) as compared by un-injured (0.72) with 0.0371 and 0.0739 SE and CV for comparison. Whereas, the interaction between treatment and method represents that there were no significant difference between un-inoculated injured, un-injured control (didn't show symptoms) and Streptomycin un-injured and injured (showed minimum 0.05 and 0.13 spots size), statistically followed by Penicillin-5 un-injured (0.40), Pencilin-1 un-injured (0.86) and Zampicilin un-injured (1.11) (Fig. 4). Significantly maximum spot size (2.43) was recorded in injured and inoculated control followed by un-injured and inoculated control (1.9312), injured fruits inoculated and treated with Penicillin-5 (1.85), Pencilin-10 (1.70) and Zampicilin (1.59) (Fig. 4). The SE and CV for comparison were 0.0909 and 0.1810; whereas there were 8 groups in which the means are not significantly different from one another at alpha 0.05.

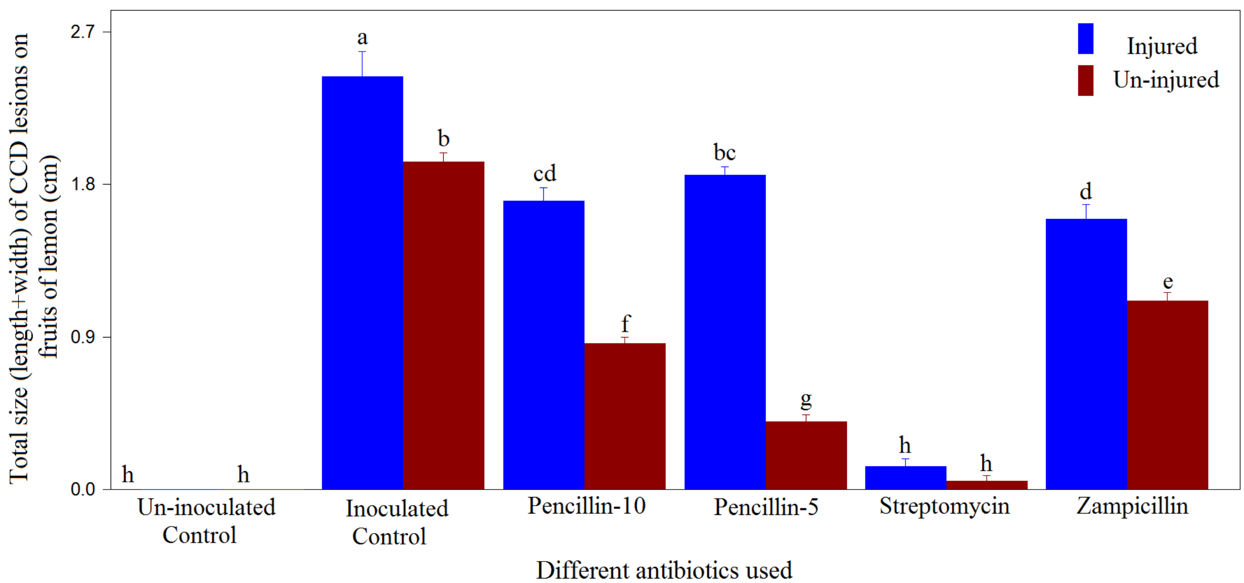

Fig. 4 Effect of different antibiotics on the total size (L+W) citrus canker lesions (cm) on fruits of lemon

***In-vitro* effect of different cheap antibiotics on CCD**

Efficacy of different cheap antibiotics for the number of citrus canker lesions per fruit

LSD for all-pairwise comparisons test of in vitro efficacy percentage of antibiotics on several spots per fruits (Fig. 5) exposed that Streptomycin found most effective antibiotic (89.07) followed by Penicillin-10 (69.22) and Penicillin-5 (63.02); while the low effective antibiotic was Zampicillin (59.76). There were no spots in un-inoculated untreated fruits. The statistical analysis represents 4 groups in which the means are not significantly different from one another and that the SE and the CV for comparison was 3.8566 and 0.7068. On the other hand, there are no significant pairwise differences among the means. The highest effect observed in un-injured (57.14) followed by injured (55.29) with 2.4391 and 4.8742 SE and the CV for comparison. Whereas the interaction between treatment and method, Streptomycin was recorded as the most effective antibiotic on un-injured and injured fruits (92.85 and 85.29), followed by Penicillin-10 injured and un-injured (70.58 and 67.85), Penicillin-5 injured and un-injured (64.28 and 61.76); while the lowest efficacy was recorded in case of Zampicillin applied on injured and un-injured fruits (58.82 and 60.71) and there was no infection (0.00) in control (even injured or un-injured un-inoculated and untreated). The SE and CV for comparison were 5.4541 and 10.899 and that there were 4 groups in which the means are not significantly different from one another at alpha 0.05.

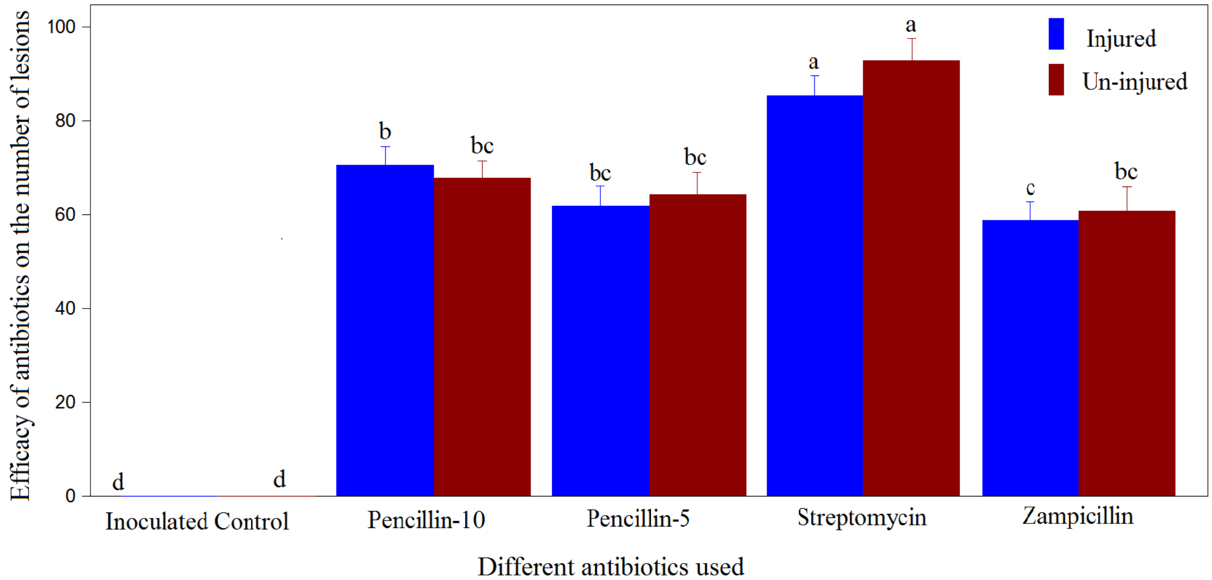

Fig. 5 Efficacy of different antibiotics on the number of citrus canker lesions on fruits of lemon

Efficacy of different antibiotics for the size of citrus canker lesions

The most effective antibiotics recorded in treatments represent Streptomycin (95.88) followed by Penicillin-5 (51.51); whereas (42.72) and (38.45) efficacy were recorded in Penicillin-10 and Zampicillin, respectively. There was no infection (0.00) in control (even injured or un-injured un-inoculated and un-treated). The SE and CV for comparison were (2.1577 and 4.3118). Whereas there were 4 groups in which the means are not significantly different from one another at alpha 0.05. However, the most effective was observed in injured (54.88) followed by un-injured (36.54), where both means are highly significantly different from one another as SE and CV for comparison was 1.3647 and 2.7270. The interaction between treatment and method showed that Streptomycin was recorded as one of the best effective antibiotics when applied in injured and un-injured (97.410 and 94.350) followed by Penicillin-5 un-injured (79.280), Penicillin-10 un-injured (55.33), the was recorded while Penicillin-5 in injured (23.74) followed by Penicillin-10 and Zampicillin injured (30.11 and 34.53) found low effective antibiotic followed by Zampicilin un Injured (42.38) (Fig. 6). The SE and CV for comparison were 3.0515 and 6.0979, whereas there were 7 groups (A, B, etc.) in which the means are not significantly different from one another at alpha 0.05.

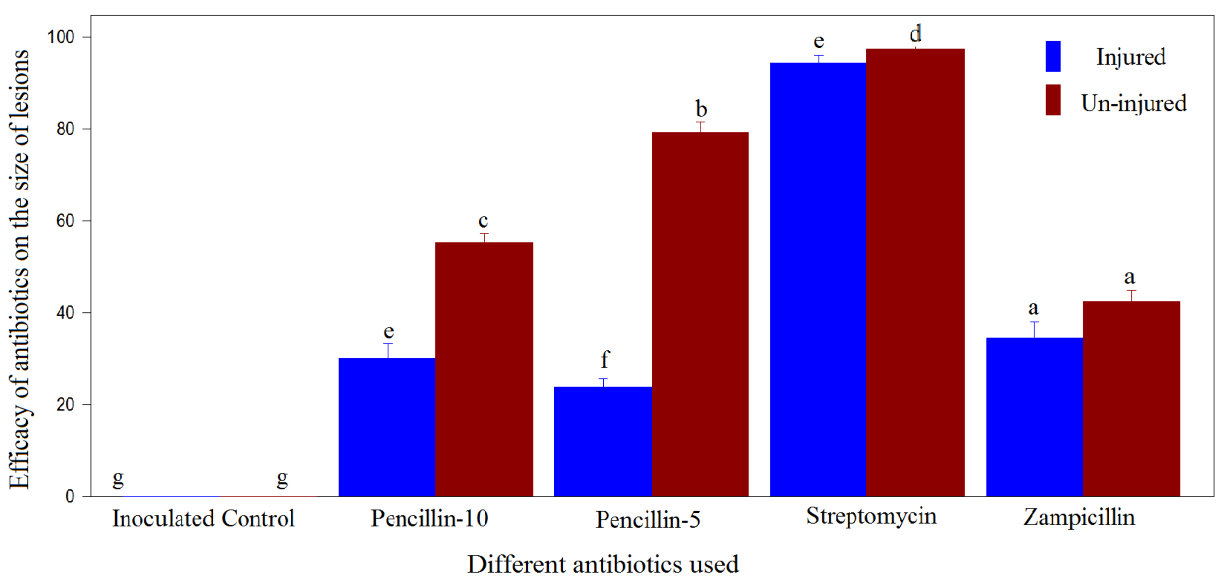

Fig. 6 Efficacy of different antibiotics on the size of citrus canker lesions on fruits of lemon

Effect of different antibiotics on disease incidence percent on fruits of lemon

There was no yellowing on fruits hence no disease incidence was recorded in un-inoculated control (0.00%), while the minimum disease percent was recorded in streptomycin (5.75%) followed by pencillin-10 (11.68%), pencillin-5 (19.06%) and zampicillin (24.62%) and the maximum disease percent was observed in inoculated control (79.00%) (Fig. 7). There were 6 groups in which the means were highly significant different from one another and SE and CV for comparison was (0.4982 and 0.9920) whereas LSD for all-pairwise comparisons test showed that maximum disease percent was recorded in injured (26.85%) followed by un-injured (19.85%) and the means of both were highly significant different from one another, where SE and the CV for comparison was 0.2876 and 0.5727. The interaction between treatment and method signifies that the LSD for all-pairwise comparisons test represents 10 groups in which means they are not significantly different from one another. There was no disease in injured and un-injured un-inoculated control (0.00%), the minimum disease percent was observed in streptomycin un-injured (2.87), followed by streptomycin injured and pencilin-10 un-injured (8.62% and 9.25%), pencilin-10 injured (14.12%), pencillin-5 un-injured (17.5%), pencillin-5 injured (20.62%) and zampicilin un-injured and injured (22.12% and 27.12%), (Fig. 7). while the maximum disease percentage observed in inoculated control injured (90.62%) followed by un-injured (67.37%) (Fig. 7). The SE and CV for comparison was 0.7045 and 1.4029.

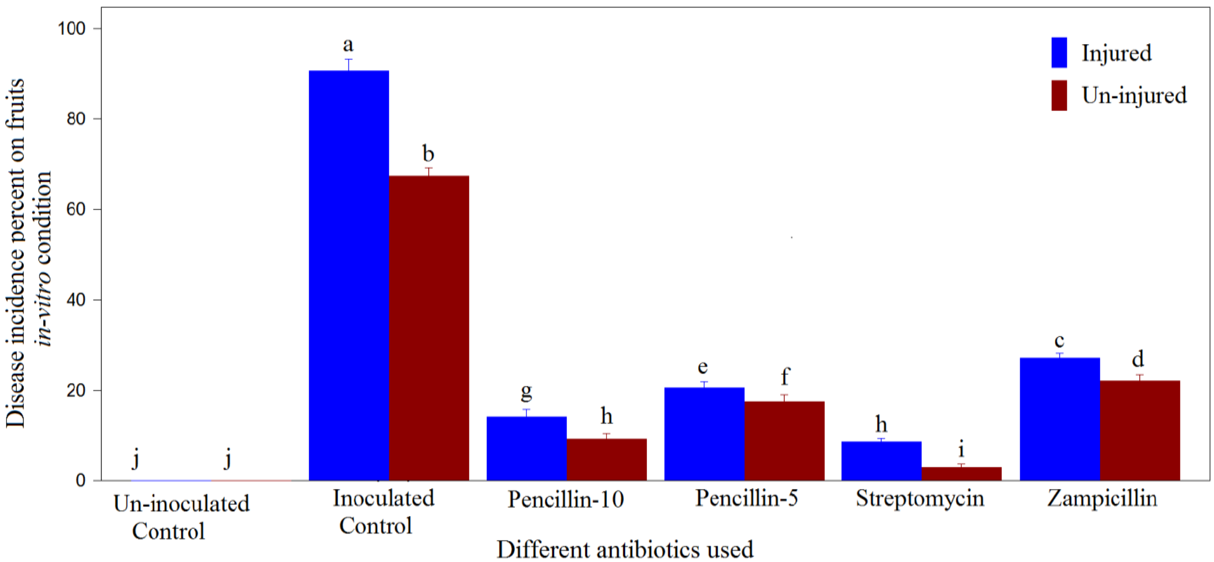

Fig. 7 Effect of different antibiotics on disease incidence percent on fruits of lemon

Discussion

Investigations on *in-vivo* and *in-vitro* management of citrus canker disease through potential and cheap antibiotics reveal that effect of different antibiotics on percent of infected leaves represents those different antibiotics found effective against the disease. Streptomycin was found to be the best followed by Pencilin-10, Pencilin-5 and Zampicilin as compared to control. Streptomycin showed (56.24%) followed by Pencilin-10 (45.83%), Penicilin-5 (39.58%) and Zampicilin (27.08%) 15 days after application, while in case of 45 days after 1st spray and 15 days after 2nd spray, the same was increased in Streptomycin (64.51%), Pencilin-10 (54.84%), Penicilin-5 (45.16%) and Zampicilin (33.87%). The effect of different antibiotics on the basis of tree directions, the results was non-significant. However, 35% efficacy was recorded on the west and north side of the trees, while in south it was (33.33%) and in east, the same was (31.66%), 15 days after 1st spray. It was increased to on the 45 days after 1st (and 15 days after 2nd) spray as (41.29%) on south, (40.00%), (40.00%) and (37.42%) on east, west and north side of the trees. The effect of different antibiotics on the percentage of infected branches represents those different antibiotics found effective against the disease. Streptomycin was found to be the best followed by Pencillin-10, Penicillin-5 and Zampicilin as compared to control. Streptomycin showed (63.33%) followed by Pencilin-10 (40.00%), Penicilin-5 (26.66%) and Zampicilin (13.35%) 15 days after application, while in case of 45 days after 1st spray and 15 days after 2nd spray; the same was increased in Streptomycin (70.59%), Pencilin-10 (55.88%), Penicilin-5 (29.41%) and Zampicilin (11.76). The effect of different antibiotics on the basis of tree directions, the results was non-significant. However, (31.99%) efficacy was recorded on the west side of the trees, while in north it was (29.33%), east, (26.68%) and in (26.66%) south, the same was (31.66%). It was increased to on the 45 days after 1st (and 15 days after

2nd) spray as (37.65%) on south and west, (30.55%) east and (28.23%) on north, side of the trees.

The effect of different antibiotics on percent of infected fruits represents those different antibiotics found effective against the disease. Streptomycin was found to be the best followed by Pencillin-10, pencillin-5 and zampicilin as compare to control. streptomycin showed (69.99%) followed by Pencillin-10 (5.00%), penicilin-5 (33.33%) and zampicilin (13.33%) 15 days after 1st spray, while in case of 45 days after 1st spray and 15 days after 2nd spray, the same was increased in streptomycin (63.33%), pencilin-10, (46.66%), penicilin-5, (26.66%) and zampicilin (16.66%). The effect of different antibiotics based on tree directions; the results was non-significant. However, (37.33%) efficacy was recorded on the north side of the trees, while in south, east and west it was (32.00%) It was increased to on the 45 days after 1st (and 15 days after 2nd) spray as (32.00%) on south and west, while the east (29.33%) and north (29.33%) side of the trees. The effect of different antibiotics on number of canker lesions/fruit shows that the minimum number of lesions were counted in streptomycin (0.43) and pencillin-10 (1.1875) followed by penicillin-5 (1.43) and zampicillin (1.56) as compared to control (3.87). There were no disease symptoms in un-inoculated and treated. Statically the result enumerates maximum lesions in injured (1.58) followed by un-injured (1.25). The data with reference to interaction between antibiotic and inoculation method represents that the minimum number of lesions were recorded in un-injured and injured fruits treated with streptomycin (0.25 and 0.62), followed by un injured fruits treated with pencilin-10 (1.12), injured fruits treated with Penicillin 10 and un injured fruits treated with penicillin 5 (1.25), un-injured fruits treated with zampicillin (1.37), injured fruits treated with pencilin-5 (1.62) and with zampicillin (1.75) as compare to control (3.5000 uninjured and (4.25) injured). There were no canker lesions in injured and un-injured control. Effect of different antibiotics on length of canker lesions on fruit demonstrated that there were no spot in un-inoculated control hence no length (0.00) was recorded but the lowest spots length was noted in streptomycin (0.05) followed by pecilllin-5 (0.55), pencillin-10 (0.67) and zampicillin (0.67). Significantly the highest spot length was recorded in inoculated control (1.10). The overall mean of inoculation methods shows that the highest spots length was observed in injured (0.64) as compare un-injured fruits (0.37). The interaction between treatment and methods represents that the lowest length was recorded in streptomycin applied in un-injured (0.02) followed by streptomycin in injured (0.07), pencilin-5 un-injured (0.17), pencilin-10 un-injured (0.44), zampicillin un-injured (0.56), zampicillin injured (0.78), pencilin-10 injured (0.90) and pencillin-5 injured (0.92), while the highest number of spots were recorded in inoculated control injured and un-injured (1.20 and 1.01), but no spots were appeared in the un-inoculated control.

Effect of different antibiotics on width of canker lesions on fruit showed that there were no spot hence no width in un-inoculated control (0.00) was recorded but the lowest spot width was found in streptomycin (0.04), followed by pecilllin-5 and 10 (0.57 and 0.60), while the highest spot width recorded in inoculated control (1.08) followed by and zampicillin (0.67). The lengthiest spots width found in injured fruits (0.63) as compared to un-injured fruits (0.35). The interaction between treatment and method showed that there was no appearance of spots in un-inoculated control injured and un-injured (0.00), the lowest spots width in the streptomycin applied on un-injured and injured fruits (0.02 and 0.62) followed by pencilin-5 un-injured (0.22), pencilin-10 un-injured (0.41), zampicillin un-injured (0.54), pencilin-10 injured and zampicillin injured 0.80 and 0.80. Significantly maximum spot width was recorded in inoculated injured control (1.23) followed by pencillin-5 injured (0.93) and inoculated uninjured control (0.92). Effect of different antibiotics on total canker lesion size (length + width) illustrated that significantly, there were no spot hence no lesion size in un-inoculated control (0.00) but was lowest in streptomycin (0.09), followed by pecilllin-5 (1.12). The highest spots size was recorded in inoculated control (2.18) followed by zampicillin and pencillin-10 (1.28 and 1.35). On an overall mean basis, there was the highest size in injured fruits (1.28) as compared by un-injured (0.72). Whereas, the interaction between treatment and method represents that there were no significant difference between un¬-inoculated injured, un-injured control (didn’t showed symptoms) and Streptomycin Un-Injured and injured (showed minimum 0.05 and 0.13 spots size), statistically followed by Pencilin-5 un-injured (0.40), pencilin-10 un-injured (0.86) and zampicilin Un-injured (1.11). Significantly maximum spot size (2.43) was recorded in injured and inoculated control followed by un-injured and inoculated control (1.93), injured fruits inoculated and treated with Pencilin-5 (1.85), Pencilin-1 (1.70) and zampicilin (1.59). Efficacy percentage of antibiotics *in-vitro* on number of spots per fruits exposed that streptomycin found most effective antibiotic (89.07%) followed by pencilin-10 (69.22%) and pencilin-5 (63.02%), while the low effective antibiotic was zampicillin (59.76%). There were no spots in un-inoculated un-treated fruits. On an overall, there are no significant pairwise differences among the means. The highest effect observed in un-injured (57.14%) followed by injured (55.29%).

The interaction between treatment and method, streptomycin was recorded as the most effective antibiotic on un-injured and injured fruits (92.85% and 85.29%), followed by pencillin-10 injured and un-injured (70.58% and 67.85%), pencillin-5 injured and un-injured (64.28% and 61.76%) while the lowest efficacy was recorded in case of zampicillin applied on injured and un-injured fruits (58.82% and 60.71%) and there were no infection (0.00%) in control (even Injured or un-injured un inoculated and un-treated). The most effective antibiotics recorded in treatments represent streptomycin (95.88% efficacy) followed by pencillin-5 (51.51%). Whereas the, (42.72%) and (38.45%) efficacy was recorded in pencillin-10 and zampicillin. There were no infections (0.00%) in control (even injured or un-injured un-inoculated and un-treated). However, the most effectiveness was observed in injured (54.88%) followed by un-injured (36.54%) where both means are highly significantly different from one another. The interaction between treatment and method showed that streptomycin was recorded as one of the best effective antibiotic when applied in injured and un-injured (97.41%) and (94.35%) followed by pencillin-5 un-injured (79.28%), pencillin-10 un-injured (55.33%), the was recorded while pencillin-5 in injured (23.74%) followed by pencillin-10 and zampicillin injured (30.11 and 34.53) found low effective antibiotic followed by Zampicilin un Injured (42.38). There was no yellowing on fruits hence no disease incidence was recorded in un-inoculated control (0.00), while the minimum disease percent was recorded in streptomycin (5.75) followed by pencillin-10 (11.68), pencillin-5 (19.06) and zampicillin (24.62) and the maximum disease percent was observed in inoculated control (79.00). The all-pairwise comparisons test showed that maximum disease percent was recorded in injured (26.85) followed by un-injured (19.85) and the means of both were highly significant different from one another. The interaction between treatment and method signifies that there were no disease in injured and un-injured un-inoculated control (0.00), the minimum disease percent was observed in streptomycin un-injured (2.87), followed by streptomycin injured and pencilin-10 un-injured (8.62 and 9.25), pencilin-10 injured (14.12), pencillin-5 un-injured (17.50), pencillin-5 injured (20.62) and zampicilin un-injured and injured (22.12 and 27.12), while the maximum disease percent observed in inoculated control injured (90.62%) followed by un-injured (67.375).

Several management practices are highlighted in the literature for the management of citrus canker disease worldwide. Kuhara, 1978; Stall and Seymour, 1983; Leite and Mohan, 1984; Leite et al., 1987; Leite, 1990; Hartung, 1992 also worked on management of citrus canker and reported that as a stand-alone measure, control of citrus canker with copper sprays on resistant or moderately resistant citrus cultivars may be achieved, whereas adequate control on susceptible or highly susceptible cultivars requires the implementation of several control measures and that usually, citrus canker is managed with preventive sprays of copper-based bactericides throughout the world. The bactericides are used to reduce inoculum build up on new leaf flushes and to protect expanding fruit surfaces from infection. However, effective suppression of the disease by copper sprays depends on several factors, such as the susceptibility of the citrus cultivar, environmental conditions, and adoption of other control measures (Kuhara, 1978; Stall and Seymour, 1983; Leite et al., 1987; Leite, 1990; Leite and Mohan, 1990; Hartung, 1992). Das (2003) stated that the citrus canker can be managed by using canker-free stock, pruning, and burning of infected plant parts before monsoon, spraying with suitable bactericides and checking the leaf miner infestation by insecticide. While, in addition, the timing and number of copper sprays for effective control of citrus canker is not only highly dependent on the susceptibility of the citrus cultivar, but on the age of the citrus trees, environmental conditions, and the adoption of other control measures. In general, 3 to 5 copper sprays are necessary for effective control of citrus canker on citrus cultivars with intermediate levels of resistance whereas, in years with weather that is highly conducive for epidemic development of citrus canker, up to 6 sprays may be recommended (Leite et al., 1987 and Leite, 1990). Zhang et al. (1996) tested several fungicides such as copper hydroxide (as Koshad), carbendazim, sulfuric acid, streptomycin and Bordeaux mixture for control of citrus canker (*X.* citri) on 6-year-old trees. The results showed that copper hydroxide gave the best disease control at 800 times concentration. Application of antibiotics can be helpful in diseases control in aquaculture and enhance the development of resistant strains of both harmful and harmless bacteria that are replacing antibiotic susceptible bacteria (Son et al., 1997; Li et al.,1999). Effective and economical chemical control has yet to be developed for this disease. This may be because the pathogen population is highly variable in its sensitivity to the antibiotics used for control (Shivalingaiah et al., 2013). Ali et al. (2017) also conducted a study on the evaluation of antibiotic susceptibility, antibacterial sensitivity and antagonistic activity through disc diffusion method against *X.* axonopodis pv. citri bacteria causing Citrus aurantifolia canker disease. Gentamycin showed highest 21.0±0.0mm diameter of zone of inhibition against the isolated bacteria, while Chethankumar et al. (2017) carried out study by using different bio agents and botanicals for management of citrus canker.

Conclusion

It could be concluded that the investigations on *in-vivo* and *in-vitro* management of citrus canker disease through potential and cheap antibiotics reveals that *in-vivo* and *in-vitro* effect of different antibiotics showed that streptomycin had the highest efficacy followed by Pencilin-, Penicilin-5 and Zampicilin However, the effect of different antibiotics on the basis of tree directions, the results were non-significant There was no disease in injured and un-injured un-inoculated control (0.00%); while the minimum disease percent was observed in streptomycin un-injured followed by streptomycin injured. The results so far achieved in the current study are hoped will be helpful for the grower in the sustainable management of CCD.

Recommendations

It could be recommended based on results regarding the investigations management of citrus canker in lemon through antibiotics that streptomycin may be used against to the disease to reduce the disease and improve the fruit quality whereas further studies on management must be planned by the researchers to find out the most easy, economical, eco-friendly and useful IPDM model against the disease.

Conflict of Interest: The authors declare that they have no conflict of interest.

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