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Research paper

Citrus rootstock characterization against citrus canker and evaluation of antibiotics effect against *Xanthomonas axonopodis* pv. *citri*

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Citrus canker (*X. axonopodis* pv. *citri*) widespread bacterial disease which limits citrus productivity and causes quality deterioration. Citrus canker is mostly a leaf-spotting and fruit rind-blemishing disease, but under highly favorable conditions, infections cause defoliation, shoot dieback, and fruit drop. Citrus rootstocks exert a high degree of influence to scions in fruit production and plant susceptibility to diseases including citrus canker. Keeping in view the importance of citrus rootstock present study was planned to evaluate the performance of citrus rootstocks against citrus canker and antibiotics response to control *X. axonopodis* pv. *citri*. Eleven exotic citrus rootstocks were selected to evaluate against canker which was inoculated by grafting. Disease index was observed after 9 months of inoculation. Five different commercially available antibiotics were selected for the *Invitro* treatment, applied at 300, 400 and 500ppm. Inhibition zone was observed at four time points (24, 48, 72 and 96 hours). Kirumakki showed maximum resistance followed by Rangpur Poona nucellar and X639 while Rough lemon was highly susceptible. In *Invitro* evaluation maximum inhibition zone was expressed by Kanamycin sulphate at 500 ppm followed by Streptomycin sulphate. Lincomycin showed minimum inhibition zone as compared with control (Distilled water, No inhibition). Biochemical analysis exhibited that the disease index (%) decrease with increase of total phenolics contents and total protein contents. Whereas disease index had increasing trend with TSS. This study concludes that according to rootstocks evaluation Kirumakki and Rangpur Poona nucellar showed resistant performance while, on other hand Kanamycin Sulphate having maximum inhabitation against canker.

Key words: citrus, biotic stresses, citrus rootstocks, scion, disease control

INTRODUCTION

Citrus production and quality have been threatened by diseases, insect pests, availability of quality irrigation water and climate change (Shafqat et al., 2021, 2020, 2019). Apart from all other problems, citrus diseases are probably the most important factor reducing production, quality and tree life around the globe (Duan et al., 2018). There is a long list of viral, fungal, bacterial and mycoplasma agents infecting citrus orchards among them citrus canker is the most important. Citrus canker is caused by the bacterium *Xanthomonas axonopodis* pv. *citri* characterized by the formation of necrotic, raised lesions on leaves, stems and fruit with raised brown water-soaked margins, usually with a yellow halo around the lesion (Gottwald et al., 2002). On heavily infected trees, citrus canker causes severe defoliation, twig dieback, general tree decline, blemished fruit and premature fruit drop (Gottwald et al., 2001) but intensity of infection varies with the species and cultivars (Falico de Alcaraz, 1986). Bacterial canker disease managed by several strategies such as essential oils of medicinal plants (Samavi et al., 2009), copper spray (Koller et al., 2006), bordeaux mixture (Dhakal et al., 1970), plant leaf extracts (Doughari et al., 2007), zinc oxide (Graham et al., 2016) and

antibiotics (Burhan et al., 2007; Sahi et al., 2007) in field and *in vitro* conditions to control the canker in citrus. Rootstocks greatly influence characters of variety as it ensures tolerance to abiotic and biotic stress conditions (Raza et al., 2016). In plants the ability to resist abiotic stress is genetically controlled and can be partial or complete. Complete resistance is mostly characterized by quality of infection and is controlled by single gene while partial resistance is characterized by quantity of infection. In complete resistance breeding approaches can successfully transfer resistant genes (Kushalappa and Gunnaiah, 2013). Meanwhile plant quarantine measures suggest the adoptability of all possible solutions to combat any limitation in productivity and quality. Apart from elimination and eradication regulatory struggles are required to control the spread of canker by using all the possible solutions such as quarantine, resistance cultivars, antibiotics or plant essential oils and plant extracts (Doughari et al., 2007). Antibiotics can control the bacterial canker disease, but the bacteria have ability to become resistant against these antibiotics. When excessive, higher doses of antibiotics are applied plant pathogens generally acquire resistance through genetic modifications. Food safety has emerged as troubling factor for current century, the chemical residues from pesticides, insecticides and fertilizers have damaging effect on human health (Carvalho, 2006). The present study was conducted to exploit antibacterial potential of exotic citrus rootstocks against canker disease based on physical and biochemical parameters and to evaluate and identify different antibiotics for the management of citrus canker disease *in vitro* by Bauer-Kirby disk diffusion method with as little dose as possible. So that new antibiotics which are more effective and citrus rootstocks with canker resistance can be recommended for cultivation.

MATERIALS AND METHODS

Plant material and experimental site

This Experiment was conducted at Institute of Horticulture Science, and Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan during 2017-18. Eleven exotic citrus rootstocks *i.e*., Rangpur Poona nucellar (*C. limonia* Osbeck), Sun chu shah (*C. reticulata* Blanco), Citrus sunki (*C. reticulata*, var. Austere*)*, Gabbuchinee (*C. aurantium* L.) , Rich 16-6 (*Poncirus trifoliata* L.), Clausena harmandiana (*Clausena harmandiana***)**, X639 (*Citroncirus spp.*), Papuan (*Citrus medica* L.), Rough lemon (*C. jambhiri* Lush), Kirumakki nucellar (*C. limonia* Osbeck), Knorr Nucellar (*C. limonia* Osbeck) were selected for evaluation against citrus canker. One-year old healthy disease-free plants grown from seeds were taken from the Citrus Sanitation Nursery,for the inoculation of bacterial pathogen (Fig 1).

Isolation and identification of bacterial pathogen (Xanthomonas campestris pv. citri)

Leaves having distinctive symptoms of citrus canker were collected in the polythene bags for the isolation of bacterium by using the dilution plate technique. Nutrient Agar medium (Khan et al., 2016) was used for isolation of bacterium. Identified organisms of *Xanthomonas axonopodis* pv. citri. were isolated, purified and multiplied. The stock culture was maintained on nutrient agar in culture tubes at 4 °C. Pieces of infected leaves portion were also directly placed on nutrient ager for pure isolation and incubated at 30 °C for 72 hours. *Xanthomonas axonopodis* pv.citri was selected as yellowish growth on media (Swings et al., 1983).

Inoculation of Xanthomonas axonopodis pv. citri on exotic citrus rootstocks

One-year old healthy plants were selected, before inoculation plants were heavily irrigated early at the morning and covered with polythene bags for 2h, for maintaining high humidity. The plants were kept under daylight for stomata opening. For inoculation lower surface of leaves were sprayed using spraying machine at maximum pressure of 1.2 kg/cm^2 till the tissue of leaves display water soaking condition, inoculum was also injected in the midrib of the leaves using 5cc needle. Simple water was sprayed in control treatment, plants were covered with polythene bags for 1 hour after inoculation humidity was maintained as required for bacterium (75-90%) described by (Basu, 1966), with water spraying twice a day (Fig 1).

Physical analysis:

Disease index (%)

Disease Index (%) was considered by counting the total numbers of leaves showing canker lesion out of total number of leaves per plant.

$$\text{Disease index (\%)} = \frac{\text{Total No. of leaves having symptoms}}{\text{Total leaves per plant}} \times 100$$

Disease rating scale was used to determine the level of resistance / susceptibility of exotic citrus rootstocks to citrus canker (Table 1).

Table 1. Disease rating scale used to determine the level of resistance/susceptibility of exotic citrus rootstocks to citrus canker

Grade	Disease incidence	Response
1	0-15	Resistant
2	16-30	Moderate
3	30-50	Susceptible
4	>50	Highly Susceptible

Weight loss (%)

The leaves samples were kept under shade for 2 days than dried in drying oven for 1 day at 62°C. Moisture loss of healthy and diseased leaves was observed by

Weight loss (%) = (Fresh weight – Dry weight) x 100

Biochemical analysis

Protein determination

Protein of the samples was determined by Bradford method (Bradford, 1976). 10 mg leaf samples were grinded with 1 ml methanol than centrifuged for 10 minutes on 170 ×g. Supernatants was separated. 20µl sample and 120µl Bradford reagent were added and incubated on room temperature for 20 minutes. Then filled in 96 wells ELISA plate and checked in biotech Eliza machine on 600 nm**.**

Total Soluble Sugars (TSS)

Rapid and convenient Anthrone reagent method was used to determine the Total soluble sugars of samples. 10 mg leaf samples were grinded with 1ml methanol than Centrifuged for 10 minutes on 170 ×g. Eight milligram of Anthron and 40 mL H_2SO_4 were mixed in 250 mL beaker and incubated at -5°C for 30 minutes. 100 µl samples and 1 ml solution

(Anthron+ H_2SO_4) in each test tube were added and heated in water bath for 1 hour at 60°C. Sample were cooled and filled in 100 µl sample in each well of Eliza plate. The plate was placed in Eliza machine and recorded the absorbance at 630 nm (Hassid and Abraham, 1957).

Fig 1. Field experiment for disease resistance evaluation in rootstocks (A) Growing of seedlings (B) seedlings transplantation in polythene bags (C) application of inoculum on citrus rootstocks for disease development

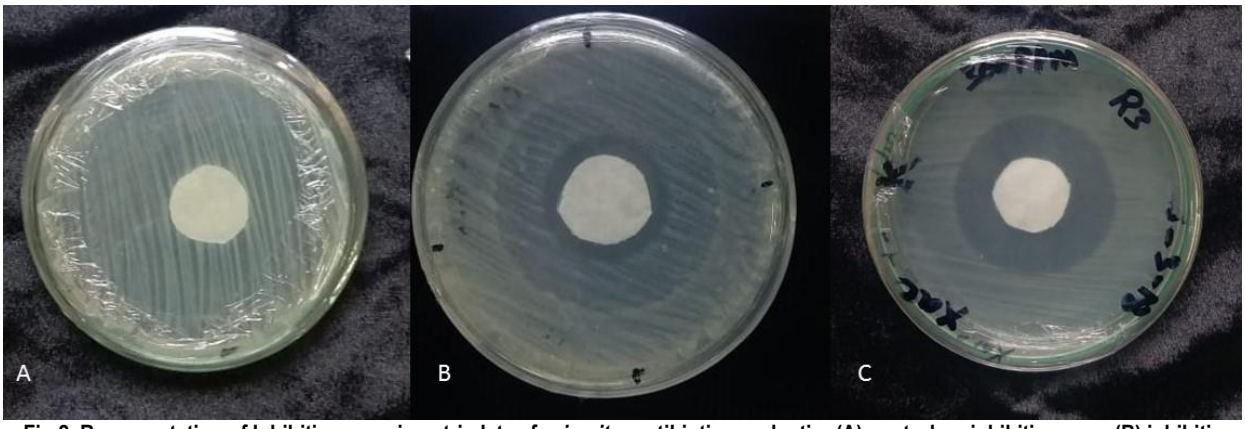

Fig 2. Representation of Inhibition zone in petri plates for *in-vitro* antibiotics evaluation(A) control no inhibition zone (B) inhibition zone at lower dose (C) inhibition zone at high dose

Total Phenolic Contents (TPC)

Total phenolic contents were determined by using Folin-Ciocalteau method which is explained by (Chaovanalikit and Wrolstad, 2004) with some modifications. Leaves were stored at (-80°C) and 10 mg leaf sample were grinded in 1mL methanol then centrifuged for 10 minutes on 170 ×g. 125 µl Na_2CO_3, FC 50 µl and 100 µl sample were added and incubated on room temperature for 2 hours than 150 µl solution were filled in 96 wells ELISA plate and checked in Biotech Eliza machine at 765 nm.

Antibiotics response against Citrus canker

In vitro evaluation of antibiotics against Xanthomonas axonopodis pv. citri

Five different antibiotics *i.e.*, Streptomycin sulphate, Kanamycin sulphate, Lincomycin, Ampicillin Sodium and Penicillin-G Benzathine were used by Bauer-Kirby method along with control (distilled water) for the experiment. Antibiotics were applied at three different concentration i.e., 300, 400 and 500 ppm against the bacterial pathogen *X. axonopodis*. The preserved bacterial suspension was streaked on new plates for further use and incubated for 48 hours at 30°C. 12 plates for each treatment were poured by NA media. The bacterial suspension was streaked into petri dishes by Swab for selected three concentrations. Filter paper disc of 22mm were dipped in solution and put in laminar air flow chamber for drying. The disc was placed in the center of media in petri dishes which were swabbed by suspension of bacterium (Barry et al., 1979). The petri dished were wrapped properly and incubated at 30ºC. The inhibition zone was observed and recorded with the help of digital Vernier caliper at four time points 24, 48, 72 and 96 hours after application (Fig 2).

Experimental Design and Statistical analysis

Experiment was laid out as Completely Randomized Design with one factor rootstocks and for second study experiment was laid out Completely Randomized Design with factorial arrangements with two factors antibiotics and concentration. All the data was analyzed by Statistix 8.1 and Graphs were constructed by SAS-Jmp 8.0. For correlation Microsoft Excel 2016 was used.

RESULTS AND DISCUSSION

Disease Index (%) and Response of citrus rootstocks against canker disease

All rootstocks showed significant disease index (%) when inoculum of *Xanthomonas axonopodis* pv. Citri was applied artificially in green house conditions to check their resistance and susceptibility against canker. Highest disease index was observed in Rough lemon (51.55%) followed by 'Knorr Nucellar' (47.22 %) and lowest disease index in Kirumakki nucellar (11.01 %) (Table 2). Disease scale showed that Kirumakki nucellar considered as resistant rootstocks against canker, whereas Rangpur Poona nucellar and X639 both rootstocks indicated as moderately susceptible. . Rest of rootstocks are susceptible to citrus canker (Table 3). The susceptibility of plant towards disease is influenced

by the genetic make up; plants with single or R gens preset for said biotic stress represent resistance while partial resistance is expressed by disease index, and lesion formation (Kushalappa and Gunnaiah, 2013).

Total Phenolic contents

Total phenolics was significantly affect by rootstocks and treatment interaction (Table 2). Higher total phenolic contents under control observed in Gabbuchinee (109.07 mg GAE/100 g FW) followed by Papuan and Rough lemon (107.91 and 100.23 mg GAE/100 g FW, respectively) and lower in Rangpur Poona nucellar (40.16 mg GAE/100 g FW). Rangpur poona nucellar showed higher phenolic contents (117.37 mg GAE/100 g FW) followed by Kirumakki nucellar (116.07 mg GAE/100 g FW) (Fig 3). Total phenolic contents and disease index (%) having inverse relation among each other. If the rootstock considered as resistant having more TPC. Kirumakki showed high phenolic contents with low disease index (%) followed by Rangpur Poona nucellar and X639. Rough lemon with high disease index (%) had low TPC (Fig 4). Kirumakki indicate overall as tolerant and rough lemon as a most sensitive rootstock on the base of total phenolic contents. Higher levels of phenolic contents observed in resistant rootstocks, because Phenolic compounds restrict pathogen invasion (Nicholson and Hammerschmidt, 1992). TPC have a significant role in controlling disease of canker in citrus plants, which were also described by (Abid et al., 2008; Raza et al., 2016). Natural ability of plants to defend against pathogenicity is commonly expressed by producing secondary metabolites *i.e.* phenolics

Table 2. Analysis of variance Sum of square for Disease index (%), Protein, Total phenolic contents, Total flavonoids and weight loss

Source	DF	Disease index %	Protein	TPC	TFC	Weight loss %
Treatment	1	14204.9**	023.23**	1297.3**	50635**	14.09**
Variety	10	3579.5**	70.95**	26578.6**	605986**	428.54**
Treatment*Variety	10	2025.8**	139.54**	18207.9**	280964**	213.11**
Error	44	1309.1	36.49	3217.2	134383	894.69
Total	65	21119.4	270.211	49301.1	1071969	1550.43

*Legend: ** indicated the highly significant results α= 5%

Table 3. Reaction of different exotic citrus rootstocks against canker disease.

Sr. #	Rootstock	Disease index (%)	Response
1	Rangpur Poona nucellar	19.71	MS
2	Sun chu shah	36.92	S
3	Citrus sunki	31.74	S
4	Gabbuchinee	34.20	S
5	Rich 16-6	35.04	S
6	Clausena harmandiana	40.69	S
7	X639	26.481	MS
8	Papuan	35.81	S
9	Rough lemon	51.55	HS
10	Kirumakki nucellar	11.01	R
11	Knorr Nucellar	47.22	S

*Legend: R=Resistant (0-15), M=Moderately Susceptible (16-30), S=Susceptible (30-50) and HS= Highly Susceptible (>50).

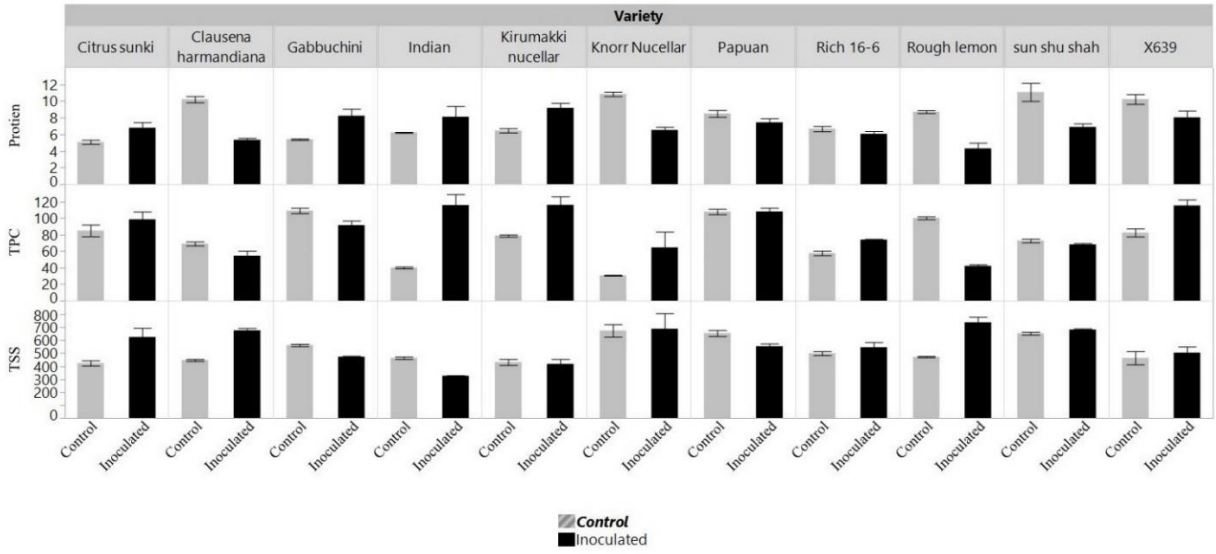

Fig 3. Total phenolic contents, Total Protein and Total Soluble Sugars and Weight loss (%) in different exotic citrus rootstocks.

which are chemically heterogeneous compounds mainly flavonoids, lignin and tannins that play a significant role in resistance against the disease (Taíz and Zeiger, 2006) also in citrus plays a significant role in resistance against citrus canker (Abid et al., 2008). Phenolics play a vital role in plant defense system against stress as these are the powerful antioxidants of plants (Sgherri et al., 2004).

Total protein content

Total protein contents were significantly affected by rootstocks and treatment interaction (Table 2). Kirumakki nucellar indicated higher protein (9.173 g/100g FW) followed by 'Gabbuchinee' and 'X639' (8.240 and 8.047 g/100g FW, respectively) under inoculated treatment. Sun chu shah (11.097 g/100g FW) followed by Knorr Nucellar and 'X639' (10.849 and 10.234 g/100g FW, respectively) had higher protein contents under control. Lowest protein contents under inoculation were observed in Rough lemon (4.307 g/100g FW) and under control in Citrus sunki (5.086 g/100g FW) (Fig 3).

Total protein content and disease index (%) had inverse relation to each other, increase in protein contents decreased the disease index (%) level in citrus rootstock. Kirumakki nucellar showed high amount of protein contents (as compared to protein in control plant) with less disease index (%) followed by Rangpur Poona nucellar. Lower protein contents and high disease index (%) was observed in Rough lemon (Fig 4). Plant pathogens like viruses, bacteria, nematode and fungi elicit synthesize of host proteins, pathogenesis related proteins which help in restricting the multiplication and spread of pathogens in healthy tissues. When plant goes under stress, it produces the protein having defensive role against the pathogens. The results supported the fact that protein having a

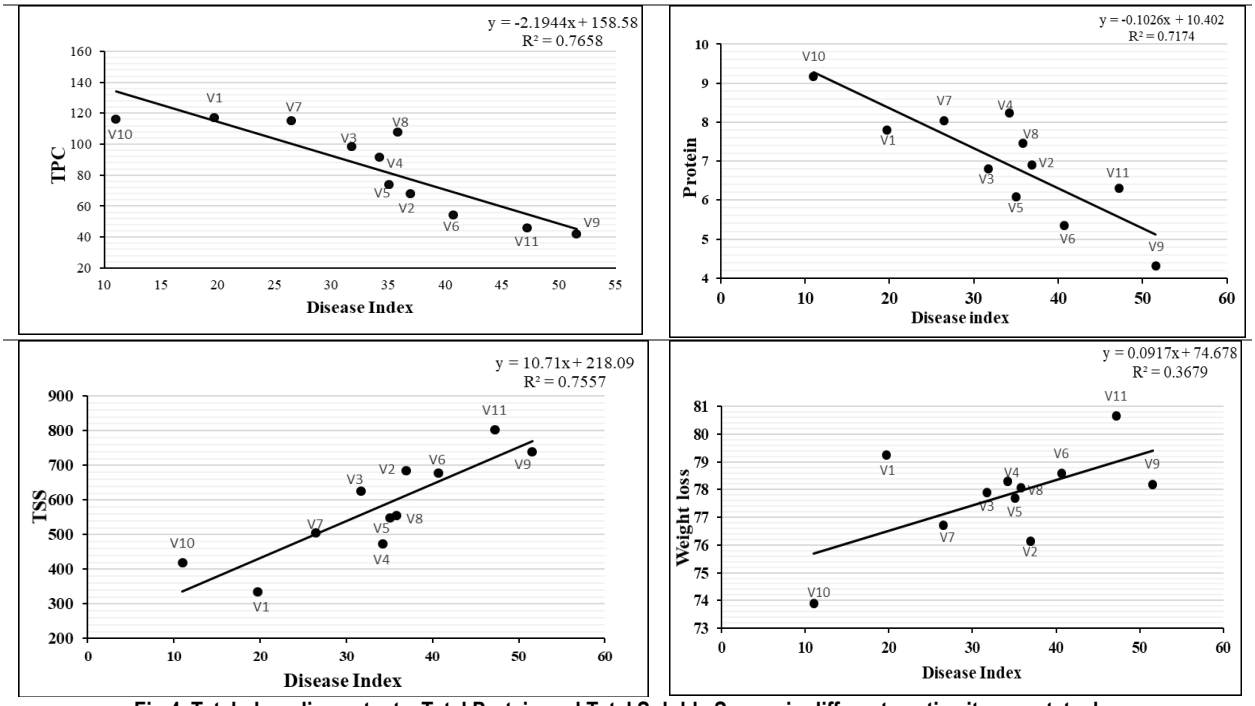

Fig 4. Total phenolic contents, Total Protein and Total Soluble Sugars in different exotic citrus rootstocks.

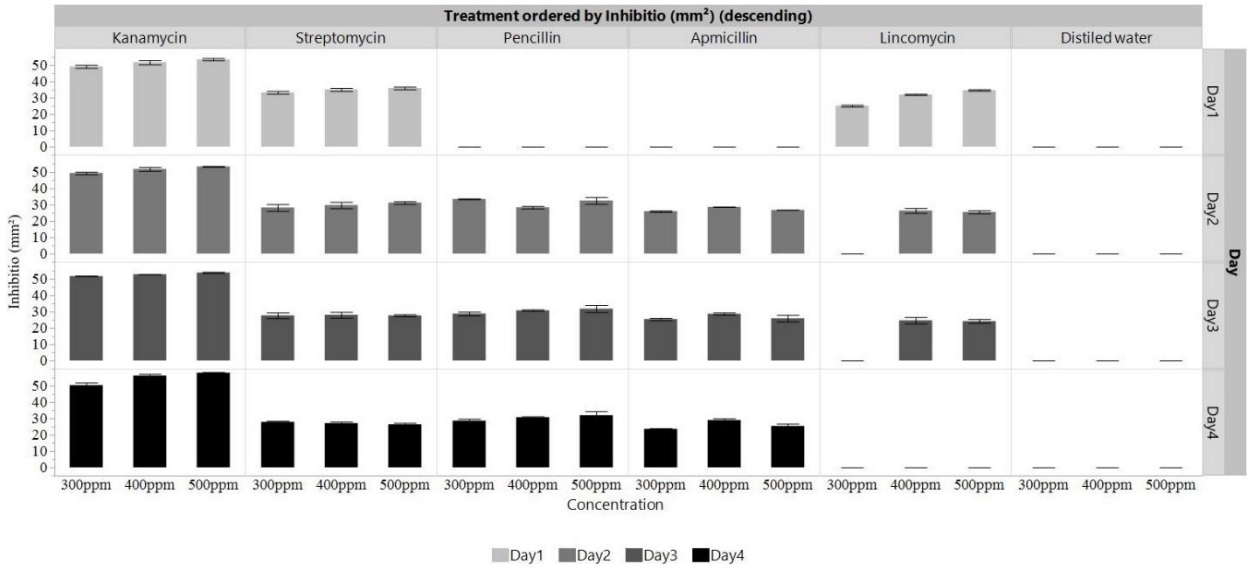

Fig 5. Inhibition zone for concentration and treat of different antibiotics against *Xanthomonas axonopodis* pv. *Citri*

significant role in controlling disease index (%) directly or indirectly by triggering any of defense system in plant, which were also discussed by (Niedz et al., 1994). Most plants synthesis protein rapidly when attacked by any pathogen. These types of proteins help to restrict the spread and multiplication of pathogen in different parts of plant and are called pathogenesis proteins. Plants have no ability to become totally immune against pathogens but they have ability to be resistance against these pathogens by a complex defense system (peptide, peroxides, ROS production) (Dangl and Jones, 2001; Sharma and Sharma, 2009; Li et al., 2020).

Total soluble solids

Rootstocks and treatments were significantly affected based on TSS (Table 2). Knorr Nucellar showed higher amount of TSS both at control (675.71) and inoculated (802.57) treatments. Lower amount of TSS in control plants were revealed in Citrus sunki (424.29) statistically at par by Kirumakki nucellar (432.38) and under inoculated in Rangpur Poona nucellar (334.29).

A positive or direct proportion of relationship observed between TSS and disease index (%). TSS were found significantly increased with plant having higher disease index (%) and low TSS with low disease index (%) in inoculated treatments as compared to control (Fig 3). Knorr Nucellar showed higher TSS with high disease index (%), whereas Rangpur Poona Nucellar low TSS with low disease index (%).

Total sugar content increased by the infection of Citrus canker. Susceptibility was positively correlated to total sugars as the total sugars were higher in the leaves of susceptible rootstocks than moderately susceptible and resistant rootstocks (Fig 4). The results support the fact that TSS having a significant role in increasing disease index (%) in citrus cultivars. Raza et al., (2016) observed that total sugars were higher in those plants which were susceptible to disease as compared to those which were resistant or moderately resistant. Total sugars have a positive relation with disease on plant leaves.

Weight loss (%) in different exotic citrus rootstocks and relationship with disease index (%)

Weight loss (%) was not significantly affected by rootstocks, treatment and their interaction (Table 2). All the rootstock behaves normally and not a clear-cut difference in weight loss (%) on both control and inoculated treatment was observed (Fig 3). Weight loss and disease index (%) showed slightly but linear trend between them. Weight loss (%) are slightly increase in those rootstocks having higher disease index (%). The results confirmed that weight loss (%) have a significant relation with disease index (%). Higher the disease index (%), higher the weight loss (%) (Fig 4).

In-vitro evaluation of antibiotics based on inhibition zone against Xanthomonas axonopodis pv. Citri

Inhibition zone indicated significant results for treatments, concentration, time and their interaction. Higher inhibition zone expressed by Kanamycin Sulphate (52.74 mm) followed by Streptomycin Sulphate (29.82 mm) no inhibition zone observed in distilled water and lower in Lincomycin (16.07 mm) *Xanthomonas axonopodis* pv. Citri. Kanamycin sulphate showed higher results after 96 hours in all 3 concentrations of 300, 400 and 500 ppm (50.46, 56.31 and 58.02 mm, respectively) as compared to 72, 46 and 24 h. Which indicated that Kanamycin Sulphate showed higher inhibition zone with increasing time (*i.e.* 48, 72 and 96 h). Streptomycin sulphate showed higher inhibition zone after 24 h in all 3 concentrations of 300, 400 and 500 ppm (33.33, 35.17 and 36 mm^2, respectively), but after that it showed decreasing trend with increasing time (*i.e.,* 48, 72 and 96 h); which indicates that *Xanthomonas axonopodis* pv. citri had little resistance against Streptomycin Sulphate after 48, 72 and 96 h. Pencillin-G Benzathine expressed no inhibition zone after 24 hours, but after 48 hours, all 3 concentration of (300, 400 and 500 ppm) express the high inhibition zone (33.57, 28.42 and 32.56 mm^2) against *Xanthomonas axonopodis* pv. citri followed by 72 and 96 h. Ampicillin sodium expressed no inhibition zone after 24 hours, but after 48, 72 and 96 hours, all 3 concentration of (300, 400 and 500 ppm) showed inhibition zone. Higher inhibition zone was expressed by concentration (400 ppm) after 48, 72 and 96 h (28.71, 28.733 and 29.093 mm, respectively), which indicated that *Xanthomonas axonopodis* pv.*citri* did not show resistance against Ampicillin Sodium till 96 h. Lincomycin showed higher inhibition zone after 24 hours in all 3 concentrations of 300, 400 and 500 ppm (25.15, 32.03 and 34.73 mm), but after that observed a decrease in inhibition zone with increasing time (*i.e.* 48, 72 and 96 h), and no inhibition after 96 hours. Which indicated that *Xanthomonas axonopodis* pv. citri showed resistance against Lincomycin after 48, 72 and 96 h. No inhibition zone was expressed by Distilled water against *Xanthomonas axonopodis* pv.*citri* (Fig 5). Application of copper-based bactericides has proven to be an effective control for management of citrus canker, theses bactericides protect the fresh growth, where disease incidence is very high and also helps to keep in check the inoculum concentration (Ference et al., 2018). The development of resistance after continuous application of bactericides forces for the development of resistance cultivars; recently CRISPER-Cas9 technology has been implied for cloning of resistant genes in commercial cultivar (Peng et al., 2017).

CONCLUSION

It is concluded from the contemporary studies that three rootstocks Kirumakki, Rangpur Poona nucellar and X639 were found resistant against citrus canker. Kanamycin Sulphate expressed maximum inhabitation with increasing time and concentration, 500ppm is regarded as good dosage for control of canker. Above mentioned rootstocks could be used as rootstocks or used in breeding programs for the development of rootstocks resistant to citrus canker. Kanamycin Sulphate can be used as antibiotic for canker control without compromising food safety and quality.

AUTHOR CONTRIBUTIONS

Kamran khan, Waqar shafqat, Sami-ur-rehman and Sufian Ikram conducted experiment and collected data. Waqar Shafqat, Muhammad Ashfaq, Sufian Ikram and Ammara Noreen performed statistical analysis and drafted the manuscript. M. Jafar Jaskani planned and supervised the experiment.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

ETHICS APPROVAL

Not applicable.

REFERENCES

Abid, M., Aslam Khan, M., & Wahid, A. (2008). Screening and determination of phenolics in relation to resistance against citrus canker. *Pakistan Journal of Phytopathology*, *20*(20.1), 109–116.

Basu, P. K. (1966). Conditions for Symptomatological Differentiation of Bacterial Canker, Spot, and Speck on Tomato Seedlings. *Canadian Journal of Plant Science*, *46*(5), 525–530.

Barry, A. L., Coyle, M. B., Thornsberry, C., Gerlach, E. H., & Hawkinson, R. W. (1979). Methods of measuring zones of inhibition with the Bauer-Kirby disk susceptibility test. *Journal of clinical microbiology*, *10*(6), 885-889.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1–2), 248–254.

Burhan, M., Sahi, S. T., & Ahmad, S. (2007). Screening of citrus cultivars for source of resistance against citrus canker under field conditions. *Pakistan Journal of Botany*, *39*(5), 1867–1871.

Carvalho, F. P. (2006). Agriculture, pesticides, food security and food safety. *Environmental Science and Policy*, *9*(7–8), 685–692.

Chaovanalikit, A., & Wrolstad, R. E. (2004). Total Anthocyanins and Total Phenolics of Fresh and Processed Cherries and Their Antioxidant Properties. *Journal of Food Science*, *69*(1), 67–72.

Dangl, J. L., & Jones, J. D. G. (2001). Plant pathogens and integrated defence responses to infection. *Nature*, *411*(6839), 826–833.

Dhakal, D., Regmi, C., & Basnyat, S. R. (1970). Etiology and Control of Citrus Canker Disease in Kavre. *Nepal Journal of Science and Technology*, *10*, 57–61.

Doughari, J. H., Pukuma, M. S., & De, N. (2007). Antibacterial effects of Balanites aegyptiaca L. Drel. and Moringa oleifera Lam. on Salmonella typhi. *African Journal of Biotechnology*, *6*(19), 2212–2215.

Duan, S., Jia, H., Pang, Z., Teper, D., White, F., Jones, J., Wang, N. (2018). Functional characterization of the citrus canker susceptibility gene CsLOB1. *Molecular Plant Pathology*, *19*(8), 1908–1916.

Falico de Alcaraz, L. (1986). Multiplication of Xanthomonas campestris pv. citri in the leaf tissue of different citrus species. *Fitopatologia*, *21*(1), 52–60.

Ference, C. M., Gochez, A. M., Behlau, F., Wang, N., Graham, J. H., & Jones, J. B. (2018). Recent advances in the understanding of Xanthomonas citri ssp. citri pathogenesis and citrus canker disease management. *Molecular Plant Pathology*, *19*(6), 1302–1318.

Gottwald, T. R., Hughes, G., Graham, J. H., Sun, X., & Riley, T. (2001). The Citrus Canker Epidemic in Florida: The Scientific Basis of Regulatory Eradication Policy for an Invasive Species. *Phytopathology*, *91*(1), 30–34.

Gottwald, T. R., Graham, J. H., & Schubert, T. S. (2002). Citrus canker: the pathogen and its impact. *Plant Health Progress*, *3*(1), 15.

Graham, J. H., Johnson, E. G., Myers, M. E., Young, M., Rajasekaran, P., Das, S., & Santra, S. (2016). Potential of nano-formulated zinc oxide for control of citrus canker on grapefruit trees. *Plant Disease*, *100*(12), 2442–2447.

Khan, N., Khan, A. A., Ahmad, M., Nouman, M., & Islam, B. (2016). Evaluation and Screening of Sweet Orange Cultivars for Vegetative Growth and Citrus Canker. *Sarhad Journal of Agriculture*, *32*(2), 121–126.

Hassid, W. Z., & Abraham, S. (1957). [7] Chemical procedures for analysis of polysaccharides. *Elsevier* 34-50.

Koller, O. C., De Oliveira, R. B. L., Nunes, D. S., Soglio, F. D., Panzenhagen, N. V., Sartori, I. A., & Manteze, F. (2006). Quemical control of citrus canker in young plants managed in conventional and organic systems. *Ciênc. Rural*, *36*(4), 1043–1048.

Kushalappa, A. C., & Gunnaiah, R. (2013). Metabolo-proteomics to discover plant biotic stress resistance genes. *Trends in plant science*, *18*(9), 522-531.

Li, Q., Qin, X., Qi, J., Dou, W., Dunand, C., Chen, S., & He, Y. (2020). CsPrx25, a class III peroxidase in Citrus sinensis, confers resistance to citrus bacterial canker through the maintenance of ROS homeostasis and cell wall lignification. *Horticulture research*, *7*(1), 1-11.

Nicholson, R. L., & Hammerschmidt, R. (1992). Phenolic compounds and their role in disease resistance. *Annual Review of Phytopathology*, *30*(1), 369–389.

Niedz, R. P. R., Doostdar, H., Mccollum, T. G., & McCollum, T. (1994). Plant Defensive Proteins and Disease Resistance in Citrus. *In Proceedings of the Florida State Horticultural Society*, 79–82.

Peng, A., Chen, S., Lei, T., Xu, L., He, Y., Wu, L., Zou, X. (2017). Engineering canker-resistant plants through CRISPR/Cas9-targeted editing of the susceptibility gene Cs LOB 1 promoter in citrus. *Plant Biotechnology Journal*, *15*(12), 1509–1519.

Raza, M. K., Khan, M. M., Naqvi, S. A., Jaskani, M. J., Mahmood, K., & Husnain, S. K. (2016). Efficacy of Citrus Rootstocks Against Citrus Canker (Xanthomonas Axonopodis Pv. Citri) Infestation. *Pakistan Journal of Phytopathology*, *28*(2), 241–247.

Sahi, S. T., Ghwzanfar, M. U., Afzal, M., Rashed, A., & Habib, A. (2007). Incidence of citrus canker disease caused by Xanthomonas campestris pv. citri (Hasse) dows on kinnow (citrus reticulata) and its chemotherapy. *Pakistan Journal of Botany*, *39*(4), 1319–1327.

Samavi, S., Hassanzadeh, N., Faghihi, M. M., & Danesh, Y. R. (2009). Effects of thyme (zaatar) essential oil and some chemical compounds in the control of citrus bacterial canker in Iran. *Journal of Plant Pathology*, *91*(3), 691–696.

Sgherri, C., Stevanovic, B., & Navari-Izzo, F. (2004). Role of phenolics in the antioxidative status of the resurrection plant Ramonda serbica during dehydration and rehydration. *Physiologia Plantarum*, *122*(4), 478–485.

Shafqat, W., Jaskani, M. J., Maqbool, R., Chattha, W. S., Ali, Z., Naqvi, S. A., Vincent, C. I. (2021). Heat shock protein and aquaporin expression enhance water conserving behavior of citrus under water deficits and high temperature conditions. *Environmental and Experimental Botany*, *181*(2021), 104270.

Shafqat, W., Jaskani, M. J., Maqbool, R., Khan, A. S., Naqvi, S. A., Ali, Z., & Khan, I. A. (2020). Genome wide analysis of citrus sinensis heat shock proteins. *Iranian Journal of Biotechnology*, *18*(4), 29–38.

Shafqat, W., Jaskani, M., Maqbool, R., & Sattar Khan, A. (2019). Evaluation of Citrus Rootstocks against Drought, Heat and their Combined Stress Based on Growth and Photosynthetic Pigments. *International Journal of Agriculture & Biology*, *22*(5), 1001–1009.

Sharma, S. K., & Sharma, R. R. (2009). Citrus canker approaching century: a review. *Tree and Forestry Science and Biotechnology*, *2*, 54–56.

Swings, J., De Vos, P., Van den Mooter, M., & De Ley, J. (1983). Transfer of Pseudomonas maltophilia Hugh 1981 to the genus Xanthomonas as Xanthomonas maltophilia. *International Journal of Systematic Bacteriology*, *33*(2), 409–413.

Taíz and Zeiger. (2006). Photosynthesis: Physiological and Ecological Considerations. *Plant Physiology*, *9*, 171–192.