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Prevalence, epidemiology and management of canker disease caused by *Xanthomonas citri* pv. *citri* in Sargodha district of Pakistan

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Abstract

A comprehensive survey was conducted during 2020 in district Sargodha (Sargodha, Sillan Wali, Bhalwal, Kot Momin, Sahiwal and Shahpur) in Pakistan to know the intensity of citrus canker. For this purpose, five orchards of each tehsil of district Sargodha were selected to find out the incidence of citrus canker by using a zigzag method of sampling. Data was recorded on five varieties (Grapefruit, Musambi, Kinnow, Khatti and Desi lemon) of citrus in all tehsils of district Sargodha. From each tehsil three orchards (10 plants of each citrus species from each orchard) were selected to collect data regarding incidence of canker. Maximum disease incidence was recorded in Sahiwal (52.5%) while the minimum in Kot Momin (29.722%). The maximum disease incidence was recorded on Grapefruit (60%). Environmental factors i.e. temperature (max. and mini ^{o}C), rainfall (mm), wind speed (km/h) and relative humidity (%) exhibited significant positive correlation with all varieties. Regression analysis showed that canker incidence (52.5%) was observed at maximum temperature (28-38 ^{o}C), minimum temperature (18-29 ^{o}C), relative humidity (40-88%), wind speed (5-15km/h) and rainfall (3-6mm). For management of citrus canker, Copper nitrate, Copper acetate, Streptomycin sulphate, Penicillin, *C. Gigentia* and Fenugreek were evaluated in lab conditions. Copper nitrate and Streptomycin were proved the most effective under laboratory conditions by expressing 33.2, 31.19 mm inhibition zone while in greenhouse and field conditions combination of copper nitrate and streptomycin sulphate expressed minimum incidence of citrus canker (13.82 and 18.44) % respectively. In case interaction between treatments and their concentration, minimum incidence of disease was expressed by combination copper nitrate and streptomycin sulphate (15.94, 13.75 11.77 and 24.41, 18.63, 12.27) % under greenhouse and field conditions, respectively. Similar observations were recorded in case of interaction between treatments and days after 7, 14 and 21 days under greenhouse and field conditions.

Keywords: Citrus canker, Environmental variables, *Fenugreek*, *Xanthomonas citri* pv. *citri*

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Introduction

Citrus is one of the most important fruit crops of Pakistan. It originated from subtropical and temperate regions of Southeast Asia (Berk, 2016). It ranks third among all fruits and is grown in more than fifty countries on commercial scale (Savita et al., 2012). It is a rich source of sugars, amino acids, organic acids, calcium, magnesium and vitamin C (Niaz et al., 2004). It is good for human health due to the presence of bioactive compounds and is an excellent detoxifier and is used for prevention of various diseases of lungs, liver, birth defects, skin cancer and heart (Durate et al., 2016). World production of citrus is about 115 million tons while production of Pakistan is 36 million tons from an area of 20.7 million hectares and occupies first position in Pakistan among all fruits (Memon, 2017). Citrus crops are threatened by a number of diseases, caused by fungi, bacteria, nematodes, viruses, phyto-plasma, and nutritional disorders that have adverse effects on the fruit quality and yield. Among these diseases, citrus canker caused by *Xanthomonas citri* pv *citri* (*Xcc*) (Hameed et al., 2020), is prevalent in citrus crop worldwide and causes huge losses every year in the world. In United States 12 million dollars were spent for its management (Zhang et al., 2011). During 2015-16, Pakistan had to face huge loss due to rejection of 144 citrus consignments due to detection of *Xcc* on citrus fruit (Pervaiz, 2015).

Xcc penetrates into the host plant through stomata, hydathodes and wounds (Ference et al., 2018). The symptom of citrus canker varies, depending on the age of the lesions, the plant part affected and the species of citrus infected (Rashid et al., 2014). Shoot lesions generally produced fewer bacteria and smaller proportion of active lesions as compared to leaf and fruit lesions (Luo et al., 2020). On leaves, symptoms appear as small, blister-like lesions, usually on the abaxial surface. With the passage of time, leaf lesions turned gray to tan brown with an oily margin, usually surrounded by a yellow halo. The center of the lesion becomes raised and corky and is visible on

both sides of the leaf. Leaf tissues in old lesions become dead and fall out. The lesions in young twigs and stem are superficially similar to those on leaves but they are generally irregular in shape. Lesions are raised with a corky appearance but there is no yellow halo. When *Xcc* enters into the host plant, it colonizes to apoplast and induces cellular hyperplasia. The proliferation of *Xcc*, at infected regions, causes raised necrotic corky lesions, which are found on leaves, stem, and fruit surface. *Xcc* is dispersed from plant to plant by strong wind and rain and is capable of producing biofilms, making it attaching to plant surfaces efficiently. Awareness about economic losses caused by citrus canker, motivate the farmers to adopt latest technologies towards canker because lack of information about control of diseases and plant protection measures are on the part of citrus growers which not only affect the production but also worth of citrus fruit (Tariq et al., 2007). To create awareness about economic losses caused by *Xcc*, a survey of citrus growing areas is very necessary because it provides a true picture about incidence and intensity of disease in a citrus growing area. That is why in the present study, a survey of potential areas of citrus with special reference to district Sargodha was conducted.

Environmental factors play a critical role in the development of plant diseases. Climate change affects the disease severity, especially citrus canker that appears in the field earlier. It is a serious issue of the citrus growing areas of Pakistan and if significant measures are not taken towards citrus canker, it is predicted that this disease will damage the citrus crop on a large scale. Farmers mostly depend on the use of chemicals which are not only causing environmental pollution but also increases cost of production. Characterization of the environmental factors provides a basic knowledge to forecast the disease in advance and create awareness among citrus growers for proper control of citrus canker (Imran et al., 20l5). Disease development is significantly influenced by environmental variables such as temperature and wind speed and relative humidity (Khan et al., 2020). For better management, knowledge of epidemiology of canker disease is necessary. That is why in the present project, the effect of different climatic factors like temperature, rainfall, relative humidity, and wind speed of district Sargodha was studied with respect to citrus canker development.

Citrus canker is managed by various strategies like use of chemicals, plant extracts or antagonists. However, chemicals are the cheapest and easiest way to manage pre- and post-harvest losses, but it may lead to environmental pollution and affect the whole ecosystem. Further the use is different; it develops resistance in bacteria against chemicals. In order to save our environment as well as human beings from the toxic effects of these chemicals, there is dire need to find out alternative ways to save our crops from these virulent pathogens (Tahir et al., 20l6). The objective of this study was to find out canker incidence, prediction of environmental factors conducive for disease development and management of citrus canker.

Materials and Methods

Incidence of citrus canker in Sargodha district

A comprehensive survey was conducted to record the incidence of citrus canker at different tehsils of Distt. Sargodha i.e., Sillan Wali, Kot Momin, Bhalwal, Sahiwal and Shahpur. Sargodha (Sargodha: latitude 32° 4' 56.8776" N and longitude 72° 40' 8.8608" E Bhalwal: latitude 32°16′19″ N and longitude 72°54′15″ E, Shahpur: latitude 32°17'11.8"N. and longitude 72°25'48.91"E. Kot Momin: latitude 32°11'24.1"N and longitude 73°1'32.77"E, Silanwali: latitude 31°49′33″ N and longitude 72°32′23″ E). Three orchards were selected and observed for recording the disease incidence in each tehsil. In each orchard, ten plants of five species of citrus (Grapefruit, Musambi, Kinnow, Khatti and Desi lemon) were observed per acre through the zigzag method (Nasir et al., 2021). Data was recorded by using following formula (Seem, 1984):

$$\text{Disease incidence (\%)} = \frac{\text{No. of infected plants}}{\text{Total no. of observed plants}} \times 100$$

Media preparation and isolation of pathogen

For the isolation of *Xcc*, infected leaves were collected and placed in brown paper bags and brought to the laboratory for further processing. Nutrient agar medium was prepared. A beaker of (1000 mL) was taken filled with 500mL distilled water and added in it 20 g agar and 13.3 g nutrient broth in a bottle. Ingredients were mixed thoroughly, the cap of bottle was tightened carefully, and autoclaved it at 121 º C and 15-psi pressure (RTA 85). In an aseptic environment (laminar flow chamber) warm media was poured in Petri plates separately. After this, samples were washed thoroughly with tap water to remove dust particles and cut into small pieces along with healthy portions and their surfaces were disinfected with 70% ethanol and placed on media plates and incubated (Heraeus) at 28±ºC for 24 hours. Light yellow colonies of bacteria were observed on samples after 24 hours. After 24 hours of incubation, bacteria were purified by using the streaking method. Bacterial isolated colonies were picked up and a single colony was multiplied on the plates for purification and then incubated at 28±ºC. Bacterium was identified on the basis of biochemical and morphological features. At 4ºC stock culture was preserved in the refrigerator at -20 °C for further use.

Pathogenicity test

For pathogenicity test, one year old, ten citrus plants of susceptible variety (Grapefruit), were purchased from nursey Institute of Horticultural Sciences. Plants were transplanted into pots containing field soil sterilized with Formalin. The bacterium from the stock culture was multiplied by incubating it at 30 °C for 48 hours on nutrient agar in an incubator. By plate count technique, aqueous suspension of the bacterium was prepared, and its concentration was adjusted @ 10^8 CFU/ mL by using spectrophotometer Before inoculation, high humidity was created by spraying and irrigation the citrus

plants and covered with polythene bags for two hours just before inoculation and placed under sunlight, so that maximum stomata become open. Using a spraying machine at a pressure of 1.1 kg / cm^2, the abaxial surface of the leaves was inoculated until the tissues showed water soaking. Control plants were sprayed with distilled water only and placed them under greenhouse conditions and data regarding disease was observed after two weeks.

Characterization of environmental factors conducive for citrus canker development in district Sargodha

Weekly environmental data comprising maximum and minimum air temperatures (°C), relative humidity (%), average rainfall (mm) and wind speed (Km/hour) were collected from Punjab Meteorological website. All the environmental and disease incidence (%) data were subjected for analysis of variance and differences in the environment factors and disease incidence, recorded on citrus varieties were determined by the least significance difference test (LSD). The influence of these environmental conditions on canker disease was determined by correlation. All these data were plotted, and the most favorable conditions were determined for citrus canker disease development by regression analysis. The varieties in which more than 50% of the environmental variables were exerting significant influences were plotted and the most favorable environmental conditions for citrus canker disease development were determined by regression analysis. Three varieties namely Grapefruit, Khatti and Desi lemon were selected for regression analysis due to presence of more disease as compared to Kinnow and Musambi.

***In vitro* evaluation of antibiotics, phyto-extracts, and chemicals against *Xanthomonas citri* pv. *citri* through inhibition zone technique**

For assessment of two antibiotics, phyto-extracts, and chemicals at three concentrations, nutrient agar media and circular pieces of 1Cm (dia.) filter paper were prepared. Media was poured into the Petri plates (9 Cm) and *Xcc* was streaked thoroughly on the media with the help of sterilized cotton swabs. Stock solution of antibiotics, plant extracts and chemicals were prepared and from stock solution three concentrations of antibiotics, plant extracts and chemicals were prepared. Pieces of filter paper were dipped into required concentration and placed in the middle of Petri plates under CRD with three replications while in case of control treatment, pieces of filter paper were dipped only in distilled paper. These Petri plates were incubated (Heraeus) at 28±ºC and data regarding the inhibition zone was recorded after 24, 48 and 72 hours.

Evaluation of effective antibiotics and chemical against citrus canker under greenhouse and field conditions

For evaluation of effective antibiotic and chemical under *in vitro* evaluation, one year old grapefruit plants were purchased from the nursery, Institute of Horticultural Sciences and brought into the greenhouse After this, plants were transferred into pots containing sterilized soil and in research area Department of Plant Pathology, University of Agriculture, Faisalabad. Effective chemicals and antibiotics with three different concentrations were evaluated alone and in combination under greenhouse and field conditions through artificial inoculation through the spraying method. Disease incidence was recorded after a 7 days interval for 3 times.

Data analysis

All the experiments in greenhouse and lab conditions were conducted under CRD while in field condition experiments were conducted through RCBD and data was analyzed by using SAS/STAT 9.4 software and means of different treatments were separated through least significant difference (LSD).at 5% probability level.

Results

Incidence of citrus canker in district Sargodha

A survey was conducted in 18 different sites of district Sargodha for the incidence of citrus canker and data was collected during September, October, November, December, January, and February with 15 days of interval during 2019-20 on five varieties of citrus (Grapefruit, Musambi, Khatti, Kinnow and Desi lemon). Maximum incidence of canker was noted on Grapefruit (49.25%) followed by Musambi, Kinnow, Khatti and Desi lemon (40.75, 37.29, 35.25 and 31.33) % respectively. Minimum incidence of canker was observed in Kotmomin (29.72) % while maximum disease incidence was noted in Sahiwal (52.5). Highest intensity of disease was noticed during November (53.05) % as compared to other months of data collection. In comparison of all sites of district Sargodha, utmost incidence of canker (55%) was noted in Chahwal, Sahiwal (Fig. 1).

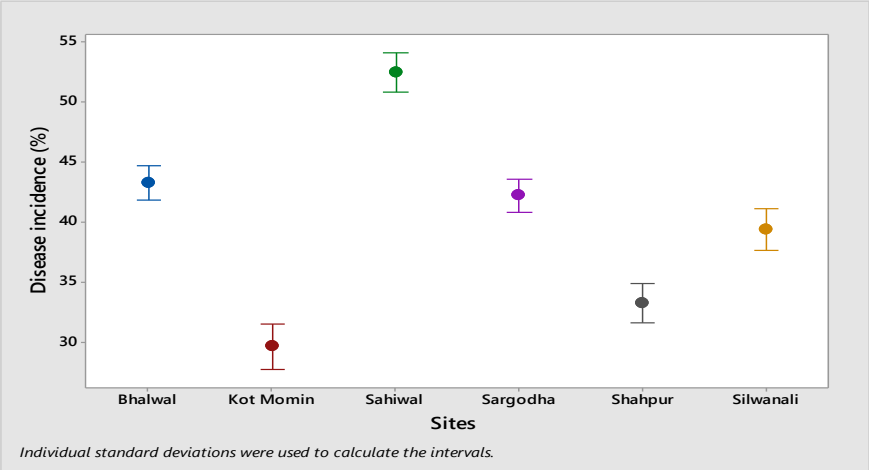

Fig. 1 Incidence of citrus canker recorded at six different tehsils of Sargodha

Correlation and characterization of environmental factors conducive for the development of citrus canker in district Sargodha

Significant positive correlation between environmental parameters (maximum temperature, minimum temperature, relative humidity, wind speed, and rainfall) and disease incidence was recorded on five varieties of citrus i.e. (Grapefruit, Musambi, Khatti, Kinnow and Desi Lemon) as indicated by P- values (Table 1). Maximum incidence of canker (52.5%) was recorded on citrus at 28 - 38 ^{o} C (max. temperature), 18-29 ^{o}C (mini. temperature), 60-88% relative humidity, 3-5.5 mm rainfall and 5-15 km/h wind speed on three varieties of citrus i.e. Grapefruit, Musambi and Khatti with high values of r as expressed in Fig. 2 A, B, C, D, E.

Table 1 Correlation between environmental factors and incidence of citrus canker on five varieties of citrus in district Sargodha

Sr. No.	Varieties	Maximum temperature (°C)	Minimum temperature (° C)	Relative humidity (%)	Rainfall (mm)	Wind speed (km/h)
1	Grapefruit	0.950**	0.995**	0.986**	0.958**	0.922**
		0.000	0.000	0.000	0.000	0.000
2	Musambi	0.915**	0.946**	0.894**	0.915**	0.838**
		0.000	0.000	0.000	0.000	0.000
3	Khatti	0.968**	0.959**	0.915**	0.926**	0.803**
		0.000	0.000	0.000	0.000	0.000
4	Kinnow	0.961**	0.973**	0.874**	0.906**	0.840**
		0.000	0.000	0.000	0.000	0.000
5	Desi	0.967**	0.971**	0.931**	0.966**	0.794**
	Lemon	0.000	0.000	0.000	0.000	0.000

** = Significant at p<0.01; Upper value indicates the Pearson's correlation coefficients; Lower value indicates the P-value.

In- vitro* evaluation of chemicals, Phyto-extracts, and antibiotics against *Xanthomonas citri* pv *citri

Among all treatments, copper nitrate showed maximum inhibition zone by copper nitrate (33.22mm) followed streptomycin (31.19mm), fenugreek (29.04mm), penicillin (28.76mm), copper acetate (27.88mm), *C. gigentia* (19.33mm) as compared to control (Fig. 3)**.** The interaction between treatments and concentrations, copper nitrate exhibited maximum inhibition zone (28.88, 32.88, 37.93mm), followed by streptomycin (26.70, 31.26, 35.60mm), fenugreek (24.73, 29.14, 33.26mm), copper acetate (24.87, 28.48, 32.93mm), penicillin (23.78, 27.88, 31.97mm), *C.gigentia* (15.52, 19.32, 23.41mm) as compared to control (Fig. 4). While interaction between treatments and duration expressed that copper nitrate exhibited maximum inhibition zone (31.82, 33.18, 34.65mm), followed by streptomycin (29.70, 31.13, 32.73mm), fenugreek (28.70, 28.51, 30.56mm), penicillin (27.35, 28.75, 30.18mm), copper nitrate (26.57, 27.76, 29.31mm), *C.gigentia* (18.16, 19.25, 20.56mm) as compared to control (Fig. 4A & B).

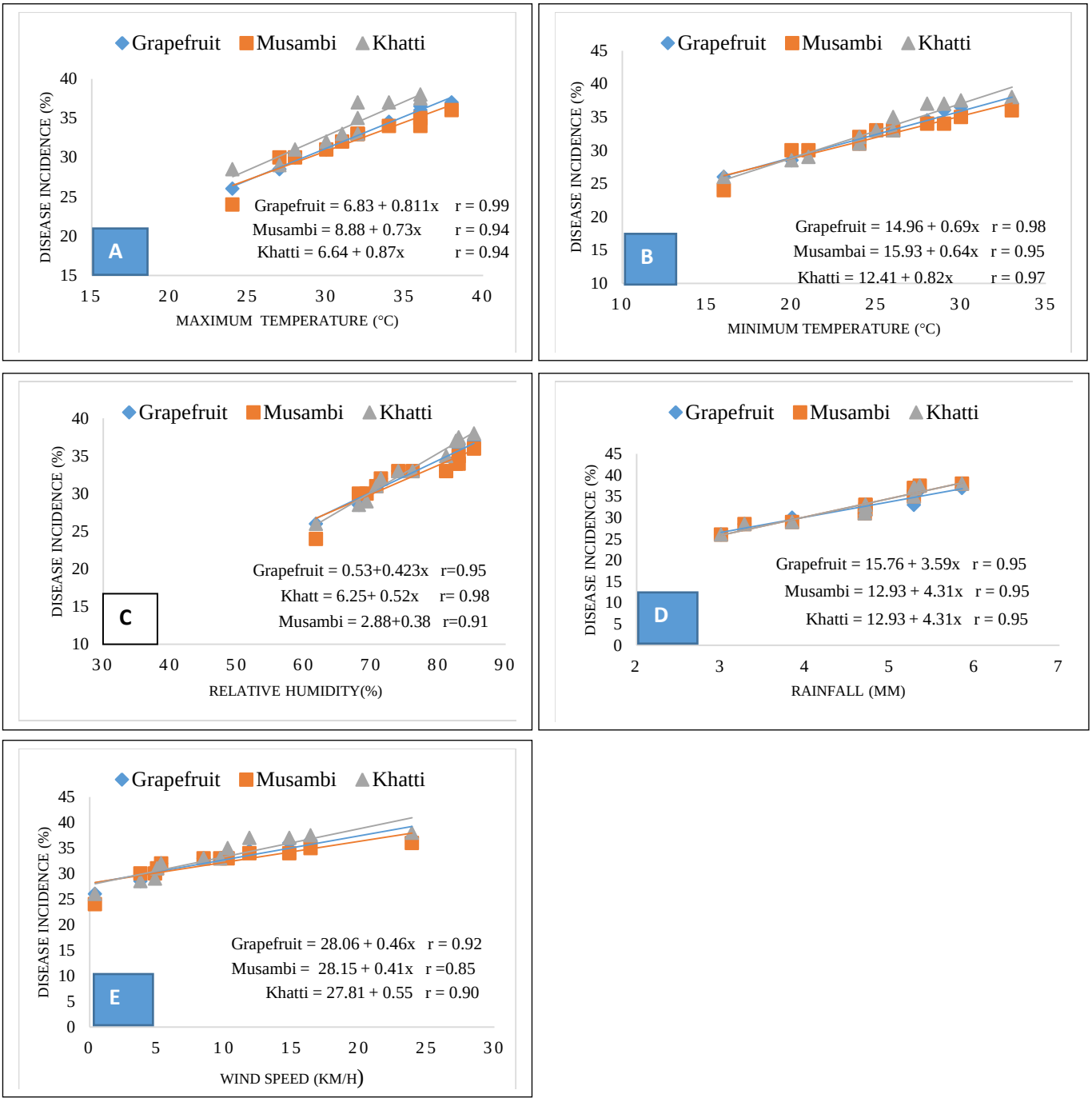

Fig. 2 Relation of maximum (A), minimum temperature (B), relative humidity (C), rainfall (D) and wind speed (E) with disease development

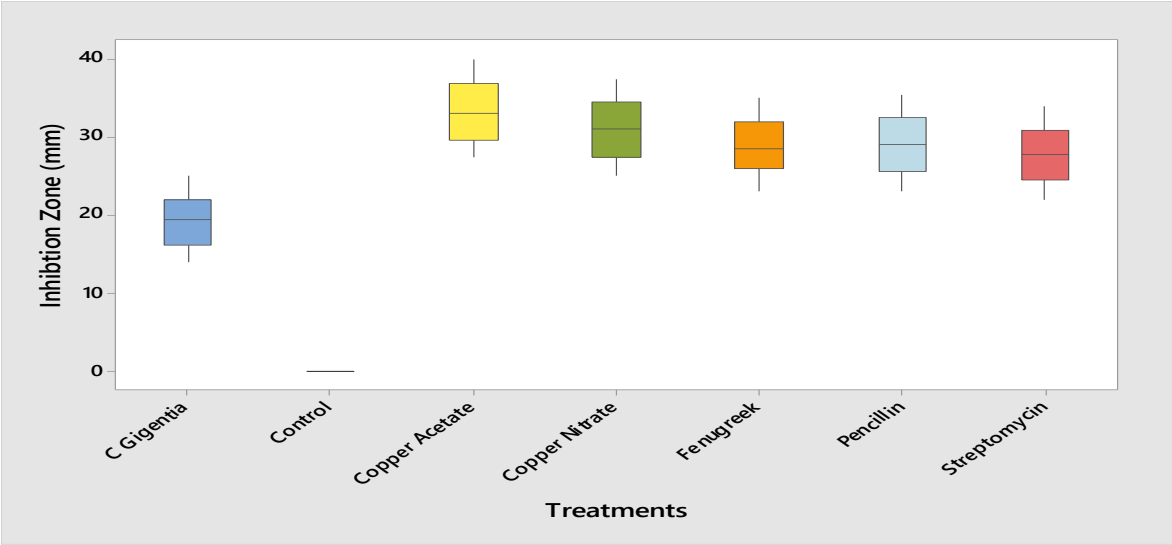

Fig. 3 *In-vitro* evaluation of phyto-extracts, chemicals and antibiotics against *Xanthomonas citri* pv *citri*.

Evaluation of copper nitrate and streptomycin under greenhouse conditions alone in combination

Among all treatments, copper nitrate + streptomycin exhibited minimum disease severity (13.82%) followed by copper nitrate (38.91%), streptomycin (38.30%), as compared to control (Fig. 5) **w**hile interaction between treatments and concentrations expressed that, combination of copper acetate + streptomycin exhibited minimum disease severity (20.43, 13.47, 7.56%) at three concentrations respectively followed by copper nitrate (43.00, 39.55, 32.36%), streptomycin (44.22, 38.12, 31.39%), as compared to control. The interaction between treatments and days indicated that combination of copper nitrate + streptomycin expressed minimum disease severity (15.94, 13.75, 11.77%) after 7,14 and 21 days respectively followed by copper nitrate (37.95, 39.69, 37.26%), streptomycin (40.20, 37.78, 35.75%), as compared to control (Fig. 6A, B).

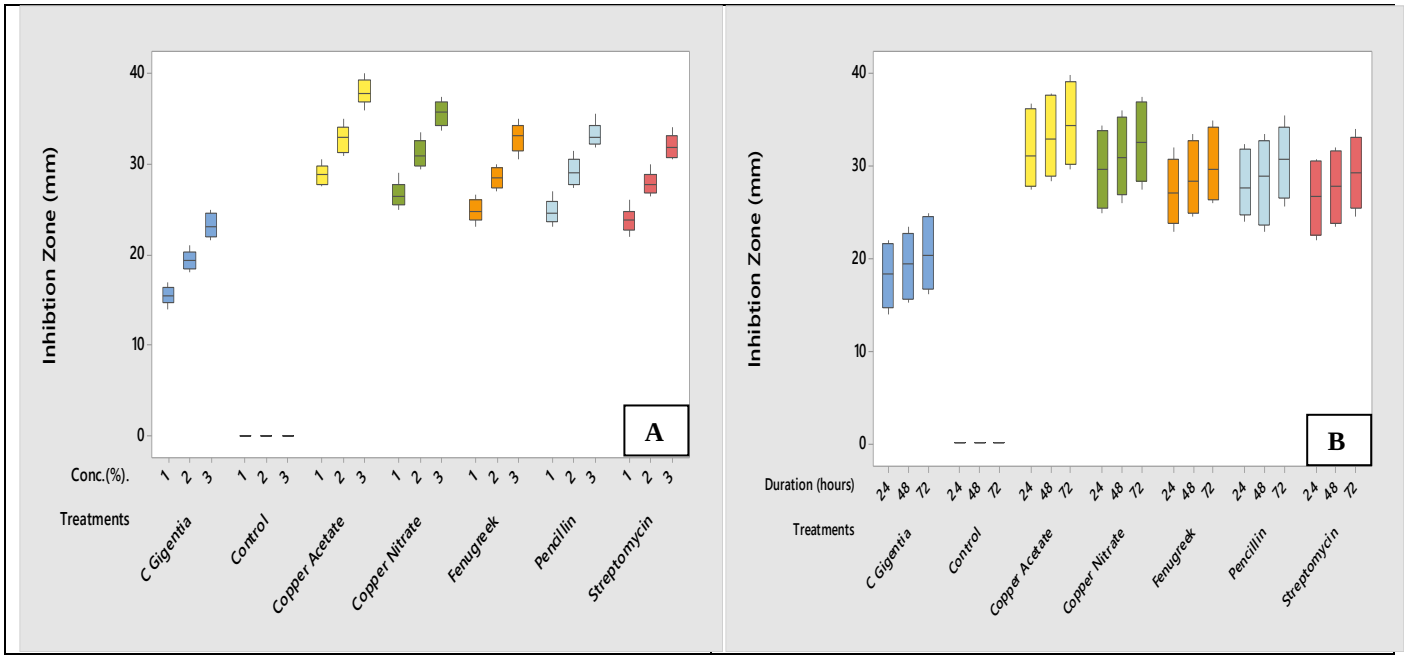

Fig. 4 Impact of interaction between treatments and concentration (A), treatments and duration (B) against *Xanthomonas citri* pv *citri*

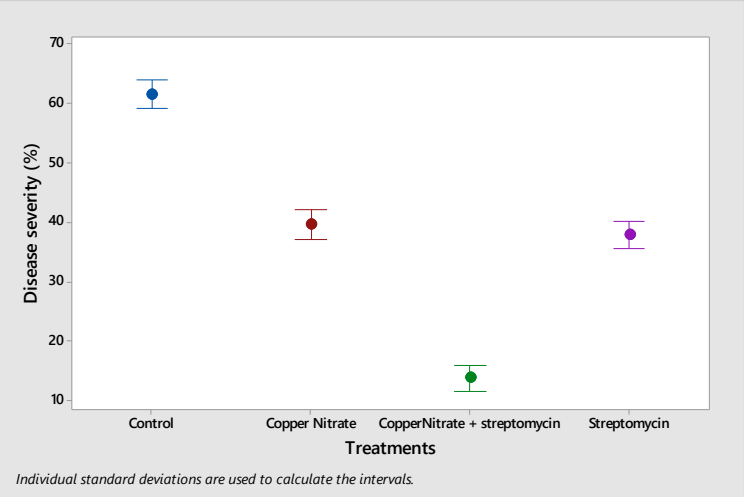

Fig. 5 Evaluation of chemicals and antibiotics against citrus canker under greenhouse conditions

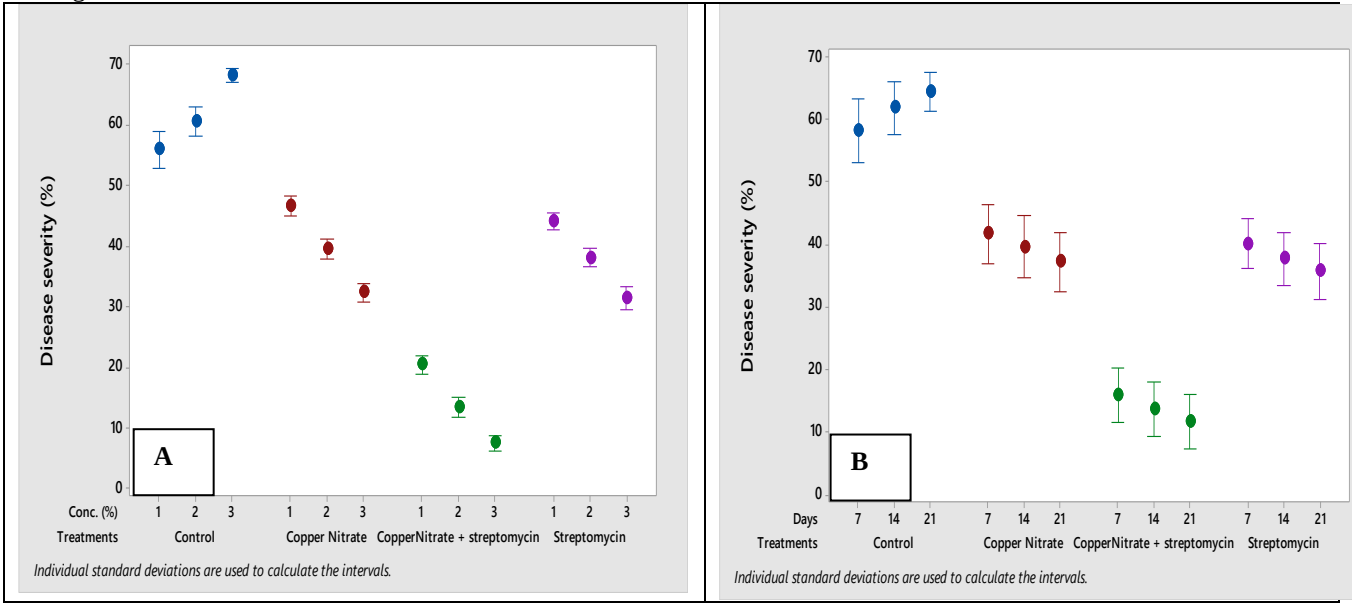

Fig. 6 Impact of interaction between treatments and concentration (A) and days, treatments, and days (B) against citrus canker in greenhouse conditions

Evaluation of chemicals and antibiotics against citrus canker in field conditions

Among all treatments, the combination of copper nitrate + streptomycin exhibited minimum disease severity (18.44%) followed by copper nitrate (24.25%), streptomycin (27.39%), as compared to control (Fig. 7)**.** While interaction between treatments and concentrations, the combination of copper nitrate + streptomycin expressed minimum disease severity (24.41, 16.63, 12.27%) at all concentration followed by copper nitrate (31.72, 23.83, 17.19%), streptomycin (34.94, 27.30, 19.92%), as compared to control. while interaction between treatments and days, the combination of copper nitrate + streptomycin showed minimum disease severity (20.52, 18.33, 16.47%) after 7,14 and 21 days respectively followed by copper nitrate (26.61, 24.38, 21.75%), streptomycin (29.87, 27.36, 24.93), as compared to control. While the interaction between treatments concentration and days exhibited that combination of copper nitrate + streptomycin expressed minimum disease incidence (26.66, 24.16, 22.41, 20.58, 18.66, 16.66, 14.33, 12.16, 10.33%) at three concentrations after 7,14 and 21days respectively followed by Copper Nitrate (34.16, 32.16, 28.83, 26.33, 23.75, 21.43, 19.33, 17.25, 15.00%), Streptomycin (37.16, 35.33, 32.33, 30.08, 26.19, 24.91, 22.38, 19.83, 17.55%), as compared to control (Fig. 8 A, B).

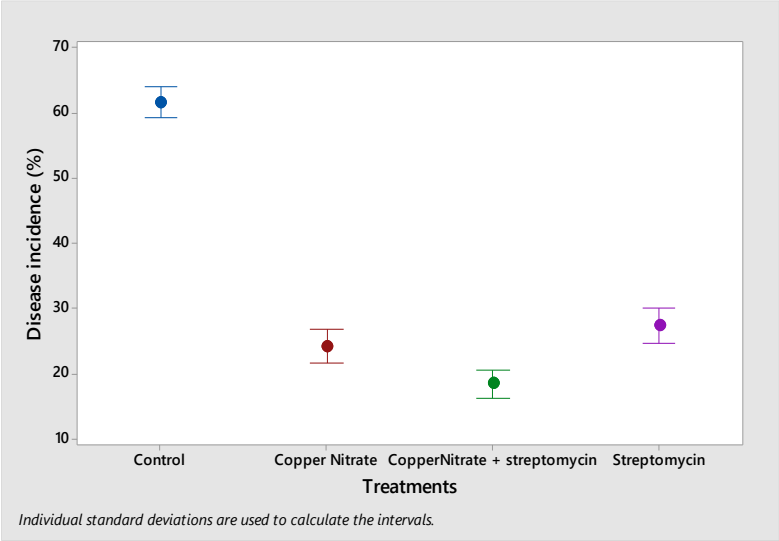

Fig. 7 Evaluation of chemicals and antibiotics against citrus canker under field conditions

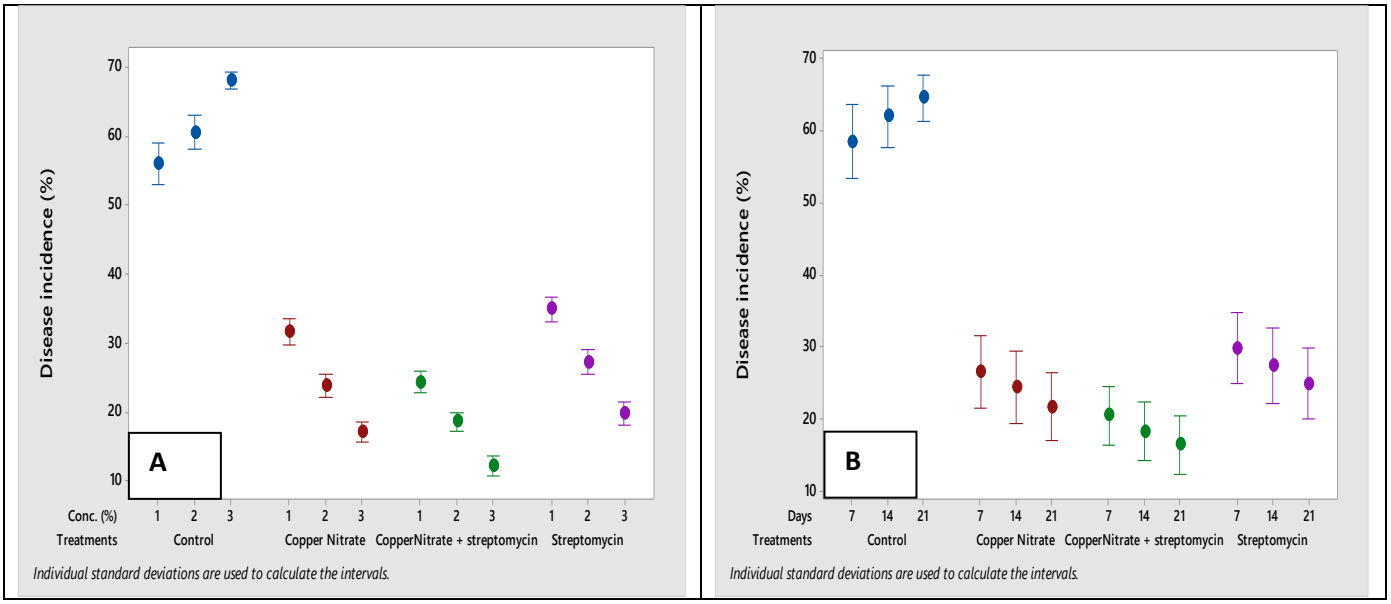

Fig. 8 Evaluation of treatments and concentrations (A), treatments and days (B) against citrus canker in field conditions

Discussion

Canker is one of the most destructive diseases of citrus. In Pakistan, canker disease caused a great decline in export of citrus fruit, causing huge economic losses to the farmers and economy of the country (Siddique & Garnevska, 2018). In the current study, a survey was conducted in district Sargodha which is hub of citrus fruit in Pakistan, to know the intensity and severity of canker. Maximum disease incidence was recorded in Sahiwal (52.5%) and minimum was recorded in Kot Momin (29.722%). These results were contradicted by the findings of (Khan et al., 2020) who found that maximum incidence of canker in Kot Momin mainly, Chak No 21 S.B and Mela while minimum disease was observed in tehsil Sargodha Chak 33 N.B. Maximum incidence of canker (52.5%) was noted during August – October. These results of this study are supported by the work of Khan et al. (2020); Khan and Abid, (2007); (Imran et al., 2015), who found maximum incidence of disease during August and September on grapefruit and musambi. This study is supported by Derso et al. (2007) who conducted a survey in eight states of Malaysia and found incidence of 36.5% and severity of 15.2% on leaves, while incidence of 18.7% and severity of 7.5% on fruits. Field host range included Mexican lime (*Citrus aurantiifolia*), pomelo (*C. grandis*) and kaffier lime (*C. hysterix*). Similarly, Burhan et al. (2007) observed the incidence of citrus canker (*Xanthomonas citri* pv. *citri*) on 15 cultivars of sweet orange (*Citrus sinensis*) in Pakistan and found all cultivars were more or less infected with citrus canker. The trend in intensity of diseased leaves and lesions per leaf was partially similar in cultivars.

As environmental factors play a crucial role in the development of canker (de Souza et al., 2021). So in contemporary study, correlation and characterization of environmental factors was done. For this purpose, the relationship between environmental factors and development of canker on five varieties (Grapefruit, Musambi, Kinnow, Khatti and Desi lemon) was studied. Significant positive correlation was observed between max, .mini. temperature, relative humidity, rainfall, and wind speed. These results are supported by the findings of Khan and Abid, (2007); Atiq, (2008) who also reported similar results. Maximum incidence of citrus canker was recorded at 28 - 38 ^{o}C (max. temperature), 18-29 ^{o}C (mini. temperature), 60-88% relative humidity, 3-5.5 mm rainfall and 5 – 15 km/h wind speed on three varieties of citrus i.e., Grapefruit, Musambi and Khatti. These results are supported by the findings of Graham et al. (2006) who reported that 28-39^{o}C temperature is optimum for pathogen development while maximum incidence of canker, at 20 – 30 ^{o}C as minimum temperature 3-6 mm rainfall (Bock et al., 2005), 60 -88 % relative humidity, 3- 5.5 Km/h wind speed (Atiq, 2008).

Many researchers reported the use of several plant byproducts, possessing antimicrobial properties, on several pathogenic bacteria and fungi (Shimpi and Bendre, 2005; Kilani, 2006). In the current study, two copper-based chemicals (copper acetate and copper nitrate), two antibiotics streptomycin and penicillin and two plant extracts *C Gigentia* and Fenugreek were evaluated under lab conditions against *Xcc.* Copper nitrate, streptomycin and fenugreek expressed significant results towards *Xcc.* While in case of greenhouse and field experiments, the combination of copper nitrate and streptomycin exhibited statistically significant results against citrus canker as compared to copper nitrate and streptomycin. These results were supported by the findings of Behlau et al. (2020) who applied different copper-based chemicals and reported significant results. Results of the current study are also in agreement with the findings of Atiq et al. (2018) who evaluated streptomycin *in vitro* and *in vivo* conditions for controlling citrus canker disease and reported significant results. Copper through different mechanisms of action cause toxicity in bacteria resulted in inhibition of protein activity, structure, function, membrane lipid peroxidation, production of reactive oxygen species and increased membrane permeability (Stevenson et al., 2013) while streptomycin cause inhibition of protein synthesis in bacteria and leads to cell death due to misreading of codon (Sharma et al., 2007).

Similar to the current study, many other researchers reported that streptomycin and CuSO4 in addition to Aureofungin solutions gave good control of citrus canker. Complete control of the canker was obtained with copper oxychloride (0.3 %). Mancozeb (0.2 %) and streptomycin + oxytetracycline (0.005 %) when sprayed at 15 days intervals, starting in early July after pruning the diseased leaves and twigs (Rehman et al., 2020). Spraying of streptomycin + copper oxychloride (3g/ L) found most effective against citrus canker (Khan et al., 2020). Integrated application of pruning of infected twigs, copper oxychloride (0.3%), streptomycin (100 ppm) and neem bark suspension were found effective. Meanwhile, copper-based chemicals are most effective against *Xcc* causing canker in citrus (Behlau et al., 2020). Other chemicals found effective against the canker were copper hydroxide (Rehman et al., 2020), mixture of Bacteriophages and ASM (Ibrahim et al., 2017), copper sulphate (Favaro et al., 2017) and combined formulations of 5 % Aureomycin and Cu or Cu -Hg.

Maximum disease incidence (52.5%) of canker was found in Sahiwal tehsil in district Sargodha, while the minimum disease incidence (29.7%) was recorded in Kot Momin. Meanwhile, among five varieties of citrus (Grapefruit, Musambi, Kinnow, Khatti and Desi lemon), maximum disease severity was recorded on Grapefruit (60%). Streptomycin and copper nitrate application control the maximum disease incidence among other treatments under greenhouse and field conditions. Early monitoring and prediction of canker disease will be helpful for farmers to manage the canker disease timely.

Conclusion

During survey of different tehsils of Distt. Sargodha maximum disease incidence was recorded in Sahiwal while the minimum in Kot Momin on Grapefruit. With increase in temperature, relative humidity and wind speed, incidence of citrus canker also increased. In case of management, minimum disease was observed when streptomycin sulphate and copper nitrate was applied in combination.

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