Research Article

Efficacy of Chemicals and Botanical Extracts to Control Citrus Canker on Kinnow in Sargodha Region

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Abstract | Citrus canker is a highly destructive malady in Kinnow mandarin worldwide. This study was conducted to determine the efficacy of both chemicals and plant-based extracts to find suitable chemicals against the canker in Kinnow. The study was carried out at the citrus farm of the Citrus Research Institute, Sargodha, Punjab-Pakistan during 2011-13 on ten-year-old bearing trees of Kinnow plants. There were nine treatments, control (no chemical was applied), Neemsol @ 5 ml, copper oxychloride @ 3 g, streptomycin sulphate @ 1 g, potassium acetyl benzoic acid @ 10 ml, difenoconazole @ 0.5 ml, extracts of *Acacia nilotica* @ 10 ml, *Datura alba* @ 10 ml, and *Allium cepa* @ 10 ml. These treatments were used by mixing in 10-liter water. The foliar spray was applied four times a year, i.e. March-April and August-September. The results showed that copper oxychloride controlled the disease up to 74%, difenconazole up to 56%, whereas, among botanicals, the disease control was 74%, 64%, 59%, and 52% due to application of extract solutions of *A. cepa*, *D. alba*, Neem Oil, and *A. nilotica*, respectively. The application of different chemicals also significantly influenced the fruit quality regarding fruit weight, fruit size, peel thickness, and juice weight. Copper oxychloride and *A. cepa* extract-treated plant revealed better results. The use of copper oxychloride, difenconazole, and extracts of *A. cepa* and *D. alba* are recommended to citrus growers to control canker in Kinnow plantations during the critical period.

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1. Introduction

Kinnow mandarin is one of the major grown cultivar in Punjab. It is grown over area of 177.22 thousand hectares with the annual production of 2116.47 thousand tons in Pakistan of which 90% is produced only in Punjab. It is therefore, vital for the economy of the country (GOP, 2018).

Export of Kinnow mandarin is only confined to traditional markets of Middle East, southeast countries, Russia and Afghanistan owing to low yields and poor quality fruit. Pakistan failed to capture higher price fetching markets mainly Europe, Australia, and America due to quality degrading diseases which result in poor yield, blemishes, and huge monetary losses. The major blemish-causing diseases in citrus are scab, melanoses, and canker which result in rejection of 60-70% of citrus consignments (Anonymous, 2020).

Among these citrus diseases, one of the serious threat is citrus canker caused by the bacterium *Xanthomonas*

axonopodis pv. citri. (Sahi *et al*., 2007; Fu *et al*., 2020). It is widely distributed disease of Indo-Pak (Das, 2003) and most commonly occurs in citrus-growing areas of Punjab (Atiq *et al*., 2007). This disease prevails in South East Asia, Japan, China, India, Pakistan and the Island of Indian Ocean (Das, 2003). The incidence of canker is common on nursery plants however infestation is also observed in orchards (Dewdney *et al*., 2016). All above ground plant parts during canker infection develop superficial raised, corky and necrotic lesions on the diseased parts that do not penetrate far into the rind and therefore fruit remains unaffected internally. However, in severe infection the plants suffer from defoliation, and dieback, which finally results in deformed fruit, and also premature fruit drop (Ference *et al*., 2018; Savietto *et al*., 2018).

Citrus canker is mainly controlled by the application of various chemicals such as fungicides and bactericides at different times of the year. Various chemicals such as copper oxychloride (Khan *et al*., 2018), streptomycin sulphate (Graham and Myers, 2011), streptomycin+oxytetracycline (Graham *et al*., 2008), streptomycin in combination with copper oxide (Graham *et al*., 2008), sodium arsenate and copper sulphate have been found effective in controlling citrus canker in different citrus growing areas (Behlau *et al*., 2008). Understanding the ecological conditions of citrus canker proliferation, are also very important aspect (Riasat *et al*., 2020). Genome editing has been started for those genes which are sensitive to citrus canker (Jia *et al*., 2017).

Extensive and repeated use of pesticides has developed resistance in plant pathogenic bacteria (White *et al*., 2002). In order to avoid the deleterious effect of synthetic pesticides on the environment, an alternative approach for the control of plant pathogenic organisms is important to tackle this problem (Khan *et al*., 2018). Mahajan and Das (2003) reported plants and microbes as a potential source of pesticide for future use. Many researchers have also reported plant by-products as anti-microbes against several plant pathogenic bacteria and fungi (Shimpi and Bendre, 2005; Kilani, 2006). Therefore, it is important to evaluate the efficacy of plant-derived products along with synthetic chemicals to curb the citrus canker. Keeping this in view, this study was conducted to evaluate and compare the efficacy of synthetic and plant extracted chemicals for the control of citrus canker in the field conditions.

2. Materials and Methods

2.1 Study area

The trial was conducted during 2011-13 at 10 years old Kinnow orchard of Citrus Research Institute, Sargodha (Punjab-Pakistan) showing clear citrus canker symptoms.

2.2 Layout

There was one plant in each replication and total numbers of plants were thirty-six. Treated and non-treated were arranged in a randomized complete block design (RCBD) with four replications.

2.3 Synthetic and plant extracts used

In this study, four synthetic chemicals and four plant based extracts were used to evaluate their efficacy against citrus canker. The detail of synthetic chemicals and plant extracts has been given in Table 1.

2.4 Preparation of plant extracts and spray solutions

Extracts from three plants *A. nilotica*; *D. alba* and *A. cepa* were used in this study. An aqueous crude extract was prepared by grinding 200 g of fresh leaves in 1000 ml of sterile distilled water with a homogenizer. The homogenate was passed through two layers of cheese-cloth and filtrate was stored at 4°C in refrigerator. Similarly, for other plant parts, 200 g for fresh and 100 g for dry samples were soaked in 100 ml of sterilized distilled water and then passed through sieve. The supernatant was used for the spray against citrus canker. The solutions of other chemicals were prepared by mixing the definite amount with tap water.

2.5 Application of spray solutions

All the chemicals and plant extracts were sprayed four times during the year in the months of March, April, July and August. Each treatment was sprayed on four plants with knapsack sprayer (20 L capacity). Control plants were left without any spray.

2.6 Disease incidence

Data was recorded after 20 days of last application from the leaves (30 leaves per plant which were taken randomly) and assessed for disease incidence (% age of canker infected leaves). A single plant was considered as one replication and a total of four plants were assessed to calculate disease incidence against each treated chemical.

The disease incidence (%) was calculated as:

$$Disease\ incidence\ (\%) = \frac{No.of\ leaves\ sampled}{No.of\ infected\ leaves} \times 100$$

Total soluble solids (Brix) were measured with digital refractometer (ATAGO, RX5000, USA) by placing 1-2 drops of juice on the prism of refractometer.

2.7 Physico-chemical analysis of citrus fruits

Kinnow fruits from treated and non-treated (control) plots were analyzed for their physical and chemical features (fruit weight, fruit size, peel thickness, juice weight, peel weight, rag weight and TSS) after harvesting each year and data of each year was pooled to obtain mean values.

2.8 Statistical analysis

The raw data was processed in MS Excel and subjected to statistical analysis by STATISTIX software version 8.1 using RCBD layout to get the ANOVA. The mean values were compared by least significance difference (LSD) test and were considered to differ significantly at $\alpha \leq 0.05$.

3. Results and Discussions

In 2011, the disease incidence (%) was significantly lower in citrus plants treated with copper oxychloride (24.17±1.60) and *A. cepa* extract (23.33±2.36) compared to control where chemical was not applied (87.50±2.85), however, effect of both copper oxychloride and *A. cepa* extract was statistically similar in suppressing the citrus canker. The significantly higher disease incidence in treated plants was found with potassium acetyl benzoic acid (70.83±2.10) and streptomycin sulphate (60.00±3.04). Among the plant based chemicals, the significantly higher disease incidence (%) was found with *A. nilotica* (48.33±2.15) (Table 1).

In the year 2012, significantly lower canker incidence (%) was found with copper oxychloride (25.00±3.19), *D. alba* extract (33.33±3.60) and *A. cepa* extract (24.17±2.85) compared to control treatment where canker disease incidence (%) was significantly higher (90.00±1.36) as compared to all other treatments. Among the chemically treated plants, citrus canker disease incidence (%) was significantly higher for potassium acetyl benzoic acid (70.00±3.04) and streptomycin sulphate (58.33±2.89) (Table 2).

In the year 2013, significantly higher canker incidence (%) was found in citrus plants not exposed with any plant or synthetic chemical (89.17±5.34) as compared to all treated plants. However, in the chemically treated plants, canker incidence (%) was significantly lower for copper oxychloride (26.67±3.60), *A. cepa* extract (25.83±0.83) and *D. alba* extract (32.50±1.60) as compared to all other treatments, but there was not significant difference in these three treatments. Again, streptomycin sulphate and potassium acetyl benzoic acid failed to suppress the disease significantly, rather a high canker incidence (%) of 61.67±0.96 and 70.83±1.60, was found, respectively, despite the application of these chemicals (Table 2).

In plots, where copper oxychloride and *A. cepa* extract were sprayed, the canker incidence (%) was only 25.28±0.83 and 24.45±1.87, respectively, which was significantly lower as compared to all other treatments; however, in both of these treatments it was statistically at par. In the plants, treated by synthetic after copper oxychloride, diphenaconozole performed better and canker incidence remained 43.33±1.20 which was significantly lower than canker incidence of streptomycin sulphate (60.00±1.20) and potassium acetyl benzoic acid (70.56±0.32) treated plots. The percent decrease in canker disease as compared to control was maximum with copper oxychloride (63.61%), and extracts of *A. cepa* (64.44%) and *D. alba* (55.56%). However, disease decrease over control was below 50% in all other treatments, with streptomycin sulphate 28.89% and potassium acetyl benzoic acid 18.33%, respectively (Table 3).

Physico-chemical analysis of kinnow fruits in treated and untreated plots showed that, the plots treated with copper oxychloride and *A. cepa* extract resulted in higher fruit weights (109.82 and 110.22 g, respectively), fruit size (50.66/62.60 mm and 52.77/61.78 mm, respectively), peel thickness (2.68 and 2.94 mm, respectively), and juice weight (51.70 and 50.95, respectively) (Table 4).

Citrus orchards are attacked by many diseases of which citrus canker is the destructive one worldwide (Atiq *et al.*, 2007). Citrus orchards in Pakistan also suffer from huge yield and quality losses of citrus fruit production as a result of bacterial canker infection (Sahi *et al.*, 2007). The chemical control is vital in managing the citrus canker in all citrus growing regions; therefore, in this study we have evaluated the effectiveness of different synthetic and plant-based chemicals in suppressing the citrus canker (Khan *et al.*, 2018).

Table 1: Synthetic fungicides and botanicals used in the experiment.

Chemical (Treatment)	Trade name	Manufacturer	Dose
Copper oxychloride	Blue Copper 50 WP	Syngenta	3g/L water
Streptomycin sulphate	Flare 72 WP	Kanzo AG	1g /L water
Potassium acetyl benzoic acid	Protector	Kanzo AG	10ml /L water
Diphenaconozole	Score 250 EC	Syngenta	0.5ml /L water
Neemsol	Neemsol	Stedec	5ml /L water
Acacia Nilotica extract	----	----	10ml /L water
Datura alba extract	----	----	10ml /L water
Allium cepa extract	----	----	10ml /L water
No chemical spray	----	----	----

Means sharing similar letter in a column are statistically non-significant (P >0.05) otherwise have significant difference

Table 2: Effect of different spray materials on citrus canker incidence (%) on citrus leaves in three different years.

Treatment	Years		
	2011	2012	2013
	Citrus canker incidence (%)		
Copper oxychloride	24.17±1.60 G	25.00±3.19 GH	26.67±3.60 G
Streptomycin sulphate	60.00±3.04 C	58.33±2.89 C	61.67±0.96 C
Potassium acetyl benzoic acid	70.83±2.10 B	70.00±3.04 B	70.83±1.60 B
Diphenaconozole	44.17±2.10 DE	42.50±2.50 DE	43.33±3.60 DE
Neemsol	40.00±2.36 EF	39.17±2.10 EF	38.33±2.89 EF
Acacia Nilotica extract	48.33±2.15 D	49.17±3.44 D	47.50±3.15 D
Datura alba extract	34.17±2.85 F	33.33±3.60 FG	32.50±1.60 FG
Allium cepa extract	23.33±2.36 G	24.17±2.85 H	25.83±0.83 G
No chemical spray	87.50±2.85 A	90.00±1.36 A	89.17±5.34 A
ANOVA parameters	DF = 8,24 F = 79.67 P<0.0001	DF = 8,24 F = 53.82 P = <0.00001	DF = 8,24 F = 47.80 P < 0.00001

Means sharing similar letter in a column are statistically non-significant (P >0.05) otherwise have significant difference.

Table 3: Effect of various chemicals and botanical extracts on citrus canker incidence (%) (pooled data of three years).

Treatment	Means of disease incidence (%)±SE	Disease decrease over control (%)*
Copper oxychloride	25.28±0.83 H	71.56±3.30 A
Streptomycin sulphate	60.00±1.20 C	32.25±1.71 E
Potassium acetyl benzoic acid	70.56±0.32 B	20.62±1.20 G
Diphenaconozole	43.33±1.20 E	51.25±2.55 D
Neemsol	39.17±1.23 F	55.93±2.31 C
Acacia Nilotica extract	48.33±1.32 D	45.63±1.93 E
Datura alba extract	33.33±2.40 G	62.50±2.45 B
Allium cepa extract	24.45±1.87 H	72.49±3.41 A
No chemical spray (control)	88.89±1.76 A	---
ANOVA parameters	DF = 8.96; F = 214.92; P<0.00001	DF = F= 2.145; P<0.00001

Means sharing similar letter in a column are statistically non-significant (P >0.05) otherwise have significant difference.

$$* \frac{\text{Percent disease incidence in control} - \text{percent disease incidence in chemical treated plants}}{\text{Percent disease incidence in control}}$$

Table 4: Effect of various chemicals and botanical extracts on the quality of kinnow fruit (pooled data of three years).

Treatments	Fruit weight (g)	Fruit size L / W (mm)	Peel thickness (mm)	Juice weight (g)	Peel weight (g)	Rag weight (g)	TSS (Brix)
Copper oxychloride	109.82±5.1 A	50.66±2.4 C/62.60±2.3 A	2.52±0.12 A	51.70±2.1 B	28.45±1.4 C	29.67±1.6 C	10±0.41 A
Streptomycin sulphate	101.65± 4.9C	49.58±3.1 D/60.42±2.1 C	2.68±0.15 AB	44.27±2.3 E	26.8±1.2 D	30.58±1.3 B	10±0.72 A
Potassium acetyl benzoic acid	108.3±4.3 AB	49.26±3.2 D/62.19±2.2 A	2.63±0.09 AB	52.62±3.0 A	24.45±1.7 F	31.23±1.3 A	10±0.23 A
Diphenaconozole	99.83±4.1 C	49.14±2.1 D/60.17±2.5 C	2.01±0.13C	47.05±2.3 C	25.23±2.1 E	27.54±1.2 E	10±0.34 A
Neemsol	99.13±5.2 C	50.9±2.4 C/59.22±3.0 D	2.15±0.12 B	45.30±2.8 D	25.02±1.3 E	28.80±1.4 D	10.5±0.22 A
Acacia Nilotica extract	86.825±4.4 D	48.10±2.7 E/57.09±2.4 D	1.98±0.12 C	44.45±2.4 DE	19.12±1.2 G	23.25±1.4 F	10±0.09 A
Datura alba extract	103.8±5.6 BC	51.53±2.6 B/60.75±2.0 C	2.15±0.20 C	45.4±2.3 D	30.05±1.7 A	28.35±0.9 D	10.5±0.21
Allium cepa extract	110.22±4.2 A	52.77±2.0 A/61.78±2.1 B	2.94±0.21 A	50.95±2.8 B	29.57±1.4 B	29.7±1.2 C	9±0.25 B
No chemical spray (control)	78.38±4.7 E	46.18±2.5 F/54.23±2.3 F	2.03±0.09 C	40.15±1.9 F	16.75±1.3 H	21.47±1.9 G	10±0.20 A
LSD value	5.63	0.29/0.26	0.31	1.63	0.14	0.20	0.22

Means sharing similar letter in a column are statistically non-significant (P >0.05) otherwise have significant difference.

Fungicides mainly copper based and antibiotics are key to control citrus canker and are applied at different interval of the year; however, their efficacy varies greatly in reducing the citrus canker incidence. In this study,we have applied four synthetic chemicals (copper oxychloride, streptomycin sulphate, potassium acetyl benzoic acid, and diphenaconozole) every year from 2011-2013. In all years, we found that percentage of citrus canker incidence was significantly least (<30%, average 25.28%) in plots where copper oxychloride was applied. The other chemicals viz., streptomycin sulphate and potassium acetyl benzoic acid did not result in suppression of canker incidence greatly in all the study years, the canker incidence with former was 60.00% in 2011, 58.33% in 2012 and 61.67% in 2013 (average 60%) while with later it was 70.83%, 70.00%, and 70.83% (average 70.56%) in the respective years.

Moreover, after copper oxychloride, significantly less canker incidence (<45%, average 43.33%) was observed as a result of application of diphenaconozole in citrus plots during three years of study. It showed if its doses and application frequency are optimized, copper oxychloride, diphenaconozole might have potential as anti-canker chemical. Present results are in line with the findings of Khan *et al.* (2018), who found remarkable control by copper based chemical. The other tested chemicals i.e., streptomycin sulphate and potassium acetyl benzoic acid were not proved effective in controlling the citrus canker significantly in this study Graham *et al.* (2011).

As the application of synthetic chemicals often pose serious environmental and health issues, therefore development of an alternative approach such as use of plant-based chemicals might be good option in controlling the plant diseases and many studies have shown that plant-based chemicals are viable source to suppress the plant diseases (Bylka *et al.*, 2004; Kilani, 2006). Therefore, in this study apart from synthetic chemicals, we have also tested four such chemicals (viz., Neemsol, *A. nilotica* extract, *D. alba* extract and *A. cepa* extract) against the citrus canker. It was found that in all years (2011-2013), citrus canker incidence (%) was least (23.33, 24.17, and 25.83%, respectively; an average 24.45%) with *A. cepa* extract as compared to all other chemicals. Such findings are compared with the results of Tahir *et al.* (2016) who used ten leaf extracts and found it effective in controlling citrus canker. Moreover, it was also observed that after the application of A. *cepa*, all other plant-based chemicals also lowered the disease to greater extent as compared to streptomycin sulphate, potassium acetyl benzoate and diphenaconozole. Similar to our study, Tahir *et*

al. (2016) reported that extracts of *A. sativum* and *A. indica* reduced the citrus canker incidence significantly; however, contrary to our results they found that *A. cepa* extract did not show promising effect in reducing citrus canker. It has been reported that organic elicitors activate defense mechanisms to fight against diseases and enhance quality of kinnow mandarin (Khan *et al*., 2020). In one of the previous study, streptomycin sulfate and *A. cepa* extract significantly reduced citrus canker (Atiq *et al*., 2018). Along with yield, enhancement in fruit quality in kinnow (*Citrus reticulata* blanco) by exogenous application of plant growth regulators, potassium and zinc (Ashraf *et al*., 2013). This beneficial effect of various micronutrients foliar spray on citrus fruit quality might be due to increase in activity of Superoxide dismutase and polyphenol oxidase activity (Macedo *et al*., 2020).

Conclusions and Recommendations

Among the synthetic chemicals, copper oxychloride had shown more effectiveness in controlling citrus canker followed by diphenaconozole. While, among botanicals, extract of *A. cepa* showed promising results against citrus canker. As the combination of copper oxychloride and *A. cepa* extract were equally effective; therefore, these can be recommended to citrus growers for the management of citrus canker.

Novelty Statement

Use of plant extracts for the control of disease is a step towards organic farming. Similarly, there will be no residual effects of chemicals in the kinnow fruit being exported.

Author's Contribution

Malik Abdul Rehman and Shafqat Ali executed the experiment. Muhammad Nawaz Khan supervised the experiment. Muhammad Babar Shahzad Afzal, and Mujahid Ali reviewed the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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