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COMPARATIVE EFFICACY OF DIFFERENT ANTIBIOTICS, FUNGICIDES AND BOTANICAL EXTRACT FOR THE CONTROL OF CITRUS CANCKER OF KINNOW MANDARIN

^aMalik A. Rehman*, ^aShafqat Ali, ^aMuhammad N. Khan, ^bSalman Ahmad, ^bMuhammad A. Ali

^a Citrus Research Institute Sargodha, Pakistan.

^b College of Agriculture, University of Sargodha, Sargodha, Pakistan.

ABSTRACT

Citrus canker is one of the most devastating disease of citrus crop. It is a worldwide disease, caused by bacterium *Xanthomonas axonopodis* pv. *citri*. Citrus canker incidence has been observed on most of the citrus varieties. Severe infection of the bacterium produces a variety of effects including defoliation, dieback, severely blemished fruit, reduced fruit quality and premature fruit drop. Present study was conducted at Citrus Research Institute, Sargodha during the years 2016-18 on ten years old kinnow plants. There were ten treatments including control with four replications. Spray was done four times in a year during the months of March, April, August, and September. The efficacy of different treatments was compared to control the disease incidence on leaves and fruits. It was concluded that Bordeaux mixture was the most suitable chemical to control the canker on kinnow plant and it also improved the quality of fruit in terms of fruit weight, fruit size and juice weight. Besides, copper hydroxide and onion extract were also found efficacious to control the disease. In future, these chemicals and plant extracts could be used in different combinations to control canker on different citrus varieties.

Keywords: Fungicides; Botanical extracts; Citrus canker; *Xanthomonas axonopodis* pv. *citri*; kinnow.

INTRODUCTION

It is an established fact that Pakistan fruit sector is dominated by citrus both in area and production. The province of Punjab contributes 95% in the entire citrus activity. It is grown over an area of 177.22 thousand hectares with the production of 2116.47 thousand tons. (Crop reporting services Pb. 2016-17). The overall scenario of kinnow export in the background of stringent measures on the quality characters has gone depressive. Among the most disturbing diseases citrus canker disease has emerged as major hurdle in the smooth flow of our kinnow in the supply chain of export. The disease from nursery to fruit harvest has serious repercussions not only on the healthy and disease free flushing of citrus orchard but also giving a serious set-

back to citrus export. In the background of this situation a well-conceived research trial has been initiated comparing the efficacy of different fungicide/bactericides and botanical extract.

Due to the disturbing factor of quality attributed to some of serious diseases of pathological origin fruit is badly blemished and is rejected by the export based supply chain. The major blemish causing diseases are citrus scab, melanose and citrus canker, however the citrus canker caused by the bacterium *Xanthomonas axonopodis* pv. *citri* is probably the worst enemy to the citrus plantations (Sahiet al., 2007). It is a common and widely distributed disease of Indo-Pak Sub-Continent (Ari fetal, 1964) and most commonly occurs in the citrus growing areas of the Punjab on most of the commercially important citrus cultivars (Hafiz and Sattar, 1952; Kuhara, 1978; Garnsey *et al*, 1979; Atiq *et al*, 2007). The disease is found in South East Asia, Japan, China, India, Pakistan and the Island of Indian Ocean (Das, 2008). The incidence of canker is very common on nursery plants as well as in the orchards. Symptoms are developed on all above ground parts of the plants that are leaves, twigs

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* Corresponding Author:

Email: arroufkhan@gmail.com

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and fruit. Raised, necrotic corky lesions are developed on diseased parts of the plant. In case of severe infection of the disease, defoliation, dieback, deformation of fruit and premature fruit drop may occur (Rossetti, 1977; Civerolo, 1981; Chand and Pal, 1982; Stall Seymour, 1983). Fluctuation of disease correlates with rain fall and temperature (Graham *et al.*, 1989). Its severity increases if rainfall occurs during new sprouting and early fruit development stages (Saleem and Tariq, 1992). Intensity of infection varies with the species and varieties (Folico de Alcoraz, 1986).

Various workers reported the control of citrus canker through chemicals. Kishun and Chand (1987) reported control of canker by four sprays of copper oxychloride (500ppm) after two prunings. Similarly, Balaraman and Pursotman (1981) found that six sprays of streptomycin sulfate (100ppm) along with two prunings reduced the canker in acid lime. Chakravarti and Hedge (1970) found that agrimycin was effective against the disease. Streptomycin in combination with bordeaux mixture had also been found effective in controlling the disease (Bhupendra *et al.*, 2017; Karishna and Noma, 1998). Other chemicals found effective are mixture of sodium arsenate and copper sulfate and thiram (Beniwal and Chaubey, 1976). Rehman *et al.* (2006) studied that streptomycin sulfate, trimiltox and copsykill were effective for the control of citrus canker. Similarly many researchers reported control of citrus canker by using antibiotics and plant extracts. Tahir *et al.* (2016) found streptomycin sulfate alone and in combination with *Allium sativum* and *Azadirachta indica* was very effective for the control of citrus canker. Islam *et al.* (2014) reported antibiotic chloramphenicol and plant extracts of *Allium sativum* and *Syzygium cumini* could be implicated for the disease citrus canker. Similarly, Yesmin *et al.* (2017) applied different fungicides and plant extracts for the control of canker of citrus (*Citrus limon*). They concluded that the fungicide Rovral 50 WP and neem leaf extract were found most effective in controlling the disease.

Extensive and repeated use of pesticides has developed resistance in plant pathogenic bacteria (Sundin *et al.*, 1994; Calrke *et al.*, 1997; William and Heymann, 1998; White *et al.*, 2002). In order to avoid the dangerous effect of synthetic pesticides on the environment, an alternative approach for the control of plant pathogenic organisms is very necessary (Hostettmann and Wolfender, 1997). Mahajan and Das (2003) reported plants and microbes as potential source of pesticide for future use. Many researchers reported plant by-products as anti-microbes against several plant pathogenic

bacteria and fungi (Dorman and Deans, 2000; Parameswari and Latha, 2001; Rath *et al.*, 2004; Britto and Santhil, 2001; Bylka *et al.*, 2004; Shimpi and Bendre, 2005; Kilani, 2006).

The chief objectives of the study was to develop effective pattern spray program in a cyclic manner based on an integrating approach to increase the overall impact on canker disease control strategy. The study conducted in Citrus Research Institute, Sargodha was expected to have very good and encouraging results to manage the notorious disease of canker. Use of plant extract for the control of disease is the step towards organic farming. Similarly, there will be no residual effects of chemicals in the kinnow fruit being exported. The bordeaux mixture and onion extract are easily available, economical and have long lasting effects to control citrus canker.

MATERIAL AND METHODS

Three types of the spray materials were used in this study that were plant extract, antibiotics and fungicides. An aqueous crude extract was prepared by grinding *Allium cepa* 200g for fresh and 100g for dry samples. Material was soaked in 100ml of sterilized distilled water and then passed through sieve. The supernatant was used for the spray against the citrus canker. The solutions of other chemicals and antibiotics were prepared by mixing the definite amount with tap water (Table 1). The ten treatments were: T1 (copper hydroxide, 3 g/L of water), T2 (Bacillus spp., 3 g/L of water), T3 (streptomycin+ copper oxychloride, 1+ 3 g/L of water), T4 (Bordeaux mixture, 1% (1:1::100)), T5 (validamycin, 1 mL/L of water), T6 (kasugamycin + copper oxychloride, 3 mL +3g/L of water), T7 (copper oxychloride + validamycin, 3 g + 1mL/L of water) T8 (onion extract, 10 mL/L of water), T9 (sulphur, 2.5g/L of water), T10 (control). All the chemicals, antibiotics and plant extract were sprayed with knapsack hand sprayer (20 L capacity) four times during the year in the months of March, April, July and August. Each treatment was sprayed on four plants. Control plants were left without any spray. The trial was conducted during the years 2016-18 on the 10 years old kinnow orchard of Citrus Research Institute, Sargodha (Punjab, Pakistan) showing citrus canker symptoms. Treatments were arranged in a completely randomized design with four replications. There was one plant in each replication and total numbers of plants were 40. Data were recorded after 20 days of last application from the leaves (30 leaves per plant which were taken randomly) and fruits and assessed for disease incidence (%age of canker infected leaf and fruit).

Table 1. Composition of different treatments with dosage rates

Treatments	Name of chemicals	Dose	Mode of action
T1	Copper hydroxide	3 g/L of water	Protectant
T2	Bacillus spp.	3g/L of water	Systemic
T3	Streptomycin+ copper oxychloride	1+3g/L of water	Systemic + Protectant
T4	Bordeaux mixture	1% (1:1::100)	Protectant
T5	Validamycin	1mL/L of water	Systemic
T6	Kasugamycin + copper oxychloride	3 mL+3 g/L of water	Systemic + Protectant
T7	Copper oxychloride + validamycin	3g+1mL/L of water	Systemic + Protectant
T8	Onion extract	10mL/L of water	Protectant
T9	Sulphur	2.5 g/L of water	Protectant
T10	Control	No treatment	N/A

How the data was recorded, statistical analysis were performed, this information is missing here. Similarly, for the study of effect of citrus canker on the physico-chemical characters of the fruit, the data

were recorded for the parameters like fruit weight, fruit size, peel thickness, juice weight, peel weight, TSS and acidity. The disease incidence % was calculated as:

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

Total soluble solids were measured with digital refractometer (ATAGO, RX5000, USA) replacing one or two drops of juice on the prism of refractometer.

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 $\alpha \leq 0.05$.

RESULT AND DISCUSSION

Control of citrus canker on leaves: Leaves are the prime organ of the plant involved in major plant functions e.g. photosynthesis, and respiration. Disease incidence on leaves directly affects the plant growth either vegetative growth or fruit production and ultimately leads to financial loss. In this experiment it was studied that use of

different chemicals significantly inhibited the attack of citrus canker.

During the year 2016, it was concluded that T4 was the excellent combination to control canker on leaves and 5.25 per 100 leaves were affected where T4 (Bordeaux mixture) was sprayed. T1 (copper hydroxide) and T8 (onion extract) also decreased the incidence of disease as compared to other treatments and 6 and 7.25 leaves out of hundred were affected respectively. In T2 (Bacillus spp.), T3 (streptomycin+ copper oxychloride), T5 (validamycin), T6 (kasugamycin + copper oxychloride), T7 (copper oxychloride + validamycin) and T9 (sulphur), number of affected leaves were 14.75, 6.5, 14.75, 4.75, 11.25 and 12.25 per hundred leaves (Figure 1 and Table 2). Control treatment showed maximum number of affected leaves that were 29.25 out of hundred leaves.

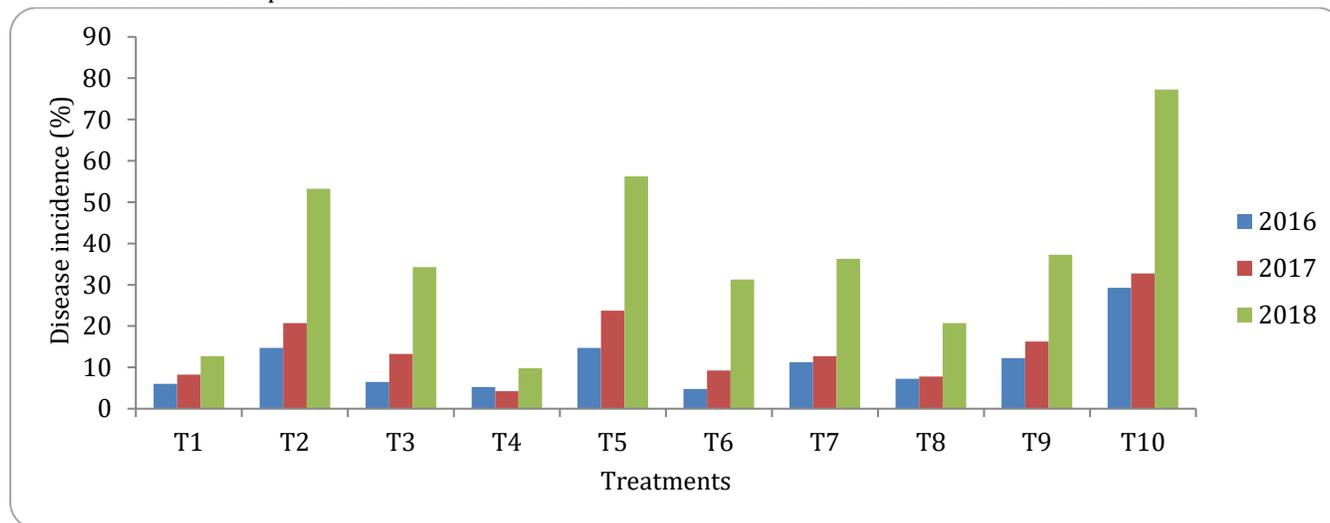


Figure 1. Effect of different treatments to control citrus canker on leaves during the year 2016-2018

Table 2. Summary of disease incidence on leaves during the years 2016-2018

Treatments	Disease incidence (%) 2016	Disease incidence (%) 2017	Disease incidence (%) 2018
T1	6 D	8.25 E	12.75 E
T2	14.75 B	20.75 B	53.25 B
T3	6.5 D	13.23 C	34.25 C
T4	5.25 D	4.25 F	9.75 E
T5	14.75 B	23.75 B	56.25 B
T6	4.75 D	9.25 DE	31.25 C
T7	11.25 C	12.75 CD	36.25 C
T8	7.25 D	7.75 EF	20.75 D
T9	12.25 BC	16.25 C	37.25 C
T10	29.25 A	32.75 A	77.25 AS

From data of 2017, T4 (Bordeaux mixture) was found very useful to control canker on leaves and minimum 4.25 out of hundred leaves were affected where T4 was sprayed. T1 (copper hydroxide) and T8 (onion extract) also reduced disease effect compared to other treatments as 8.25 and 7.75 leaves per hundred were affected, respectively. All other treatments could not produce satisfactory results and canker effect was increased gradually. In T2 (Bacillus spp.), T3 (streptomycin+ copper oxychloride), T5 (validamycin), T6 (kasugamycin + copper oxychloride), T7 (copper oxychloride + validamycin) and T9 (sulphur) plants, numbers of affected leaves were 20.75, 13.23, 23.75, 9.25, 12.75 and 16.25 per hundred leaves, respectively. Control treatment plants had maximum number of affected leaves 32.75 out of hundred leaves (Figure 1).

In 3rd year it was concluded that T4 had optimum results and minimum number of leaves were affected where T4 was sprayed as 5.75 leaves were affected per 100 leaves. T1 and T8 were also effective to control the disease as 12.75 and 20.75 leaves were affected, respectively. T2, T3, T5, T6, T7, and T9 showed number of affected leaves 53.25, 34.25, 56.25, 31.25, 36.25 and 37.25 respectively (see Figure 1). Control treatment had maximum number of affected leaves i.e. 77.25 out of hundred leaves.

Control of citrus canker on fruit: Citrus canker affects the fruit of citrus and significantly reduces fruit yield and fruit quality. Scientists had applied many control measures to control this loss. In this study, 10 different treatments were applied and finally got the following results after three years of applications.

This study demonstrated that T4 proved the excellent combination to control canker on fruit during the year 2016 and 0.5 per 100 fruit was affected where T4 was sprayed. T1 and T8 also reduced its effect compared to other treatments as 1 and 2.8 per 100 fruits were affected, respectively. In T2, T3, T5, T6, T7 and T9 number of effected fruits per hundred were 3.75, 2.5, 4, 2.9, 3.5, and 3.8, respectively (Figure 2). Control treatment had maximum number of affected fruits 6.5 out of hundred fruits.

In the second year results showed that T4 was the best to control canker on fruit, and all the fruits were found free of canker. T1 and T8 also showed positive effects and reduced the disease impact up to a certain limit as 0.5 and 0.25 per hundred fruits were damaged. In T2, T3, T5, T6, T7 and T9 number of affected fruits were 2.25, 1.25, 1.75, 0.5, 1.75 and 2.25, respectively (Table-3). Control treatment had maximum number of affected fruits that were 5.25 out of hundred (see Figure 2).

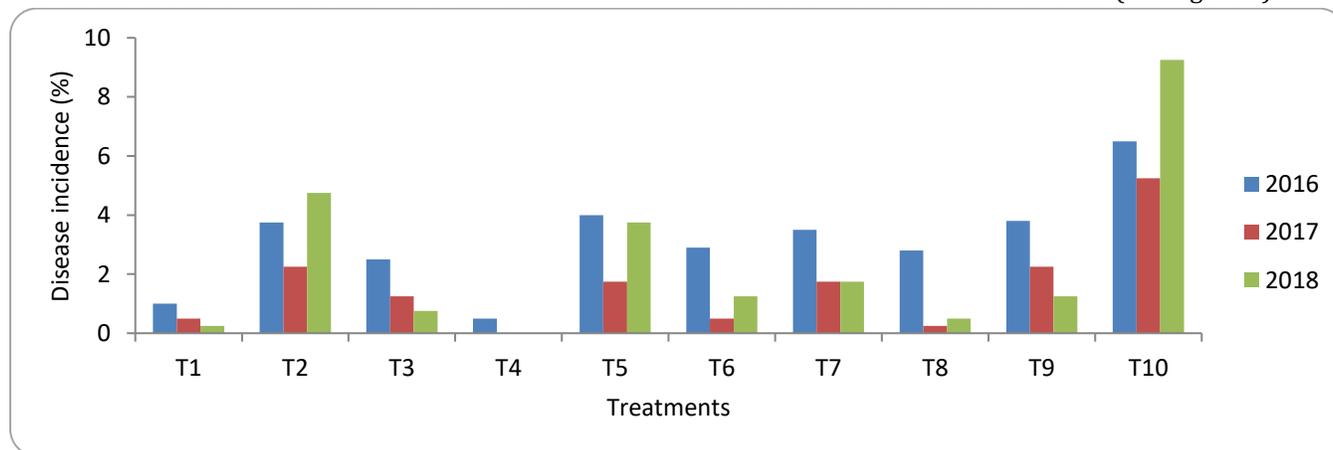


Figure 2. Comparative efficacy of different treatments to control citrus canker on fruit during the year 2016-2018

Table 3. Comparison of efficacy of different treatments to control the disease on fruit during the years 2016-2018

Treatments	Disease incidence (%)		Disease incidence (%)	
	2016	2017	2018	2018
T1	1 CD	0.5 DE	0.25 D	
T2	3.75 B	2.25 B	4.75 B	
T3	2.5 B	1.25 CD	0.75 CD	
T4	0.5 D	0 E	0 D	
T5	4 B	1.75 BC	3.75 B	
T6	2.9 B	0.5 DE	1.25 CD	
T7	3.5 B	1.75 BC	1.75 C	
T8	2.8 BC	0.25 E	0.5 CD	
T9	3.8 B	2.25 B	1.25 CD	
T10	6.5 A	5.25 A	9.25 A	

Table 4. Effect of chemicals on the fruit size and quality of Kinnow.

Treatments	Fruit wt (g)	Fruit size (mm)L/w	Peel Thickness (mm)	Juice Wt (g)	Peel wt (g)	Rag Wt (g)	TSS	Acidity
T1	163.53	61.07/75.21	3.31	65.68	45.2	52.3	9	0.72
T2	156.95	56.87/70.99	3.55	62.8	48.7	46.0	9	0.86
T3	151.37	55.98/72.66	2.98	65.08	39.05	47.25	9.5	0.89
T4	175.05	63.73/79.80	2.88	68.87	42.0	46.2	9	0.86
T5	158.35	56.75/74.23	2.72	60.55	47.83	50.67	9	0.91
T6	153.53	58.14/72.71	2.71	63.78	45.85	43.9	9	0.89
T7	141.93	53.32/68.54	2.64	60.65	39.60	41.67	10	0.87
T8	165.57	59.05/75.28	2.63	67.98	46.7	50.95	10	0.97
T9	155.50	56.05/70.81	3.12	64.87	43.75	46.88	9	0.94
T10	139.10	55.19/67.07	2.79	60.65	37.23	41.15	10.5	0.92

In the final year, T4 showed the best results to control canker on fruit and no fruit was affected with canker. T1 and T8 also reduced its affect as compared to other treatments where 0.25 and 0.5 fruits were affected, respectively. In T2, T3, T5, T6, T7 and T9, the affected fruits were 4.75, 0.75, 3.75, 1.25, 1.75 and 1.25 per hundred fruits, respectively (see Figure 2). Control treatment had maximum number of affected fruits 9.25 out of hundred fruits.

Present results are in line with the findings of Khan et al. (2018) who found remarkable control of citrus canker by copper-based chemicals. Similarly, in one of the previous study, streptomycin sulfate and *A. cepa* extract significantly reduced citrus canker (Atiq *et al.*, 2018). Such findings are comparable with the results of Tahir et al. 2016 who used ten leaves extract and found *A. cepa* effective in controlling citrus canker.

Effect of treatments on the physico-chemical parameters of kinnow: All the treatments affected the fruit quality significantly in different aspects but T4 showed good results in all parameters. Fruit parameters measured were fruit weight, fruit juice, fruit size, peel thickness, juice weight, peel weight, Rag weight, TSS and acidity. In different treatments different results were obtained but T4 (Bordeaux mixture) showed excellent

results and maximum fruit weight, fruit size, and juice weight was obtained in T4 and it was 175.05 g, 63.73/79.80 mm (L/W) and 68.87 g, respectively and minimum was in control treatment that was 139.10 g, 55.19/67.07 mm (L/W) and 60.65 g, respectively. Peel thickness, peel weight, TSS and acidity was of standard value that is required in excellent quality fruit and it was 2.88 mm, 42.0 g, 9 and 0.86 for T4. Besides T4, T1 and T8 also had shown good results.

CONCLUSION

This study was carried out to find the comparative efficacy of different antibiotics, fungicides and botanical extracts for the control of citrus canker of kinnow. This experiment was carried out at Citrus Research Institute, Sargodha during the years 2016-18 on ten years old kinnow plants. There were ten treatments including control with four replications hence total number of plants were forty. Spray was done four times in a year during the months of March, April, August, and September and data were collected after each spray. The data were analyzed and it was concluded that bordeaux mixture (T4) was the most suitable to control the canker on leaves and fruit. Copper hydroxide and onion extract were other treatments that effectively controlled disease incidence on leaves and fruit. The effect of chemicals on

the quality of fruit was investigated and it was found that bordeaux mixture significantly improved the quality of fruit including fruit weight, fruit size and juice weight. After three years of study, it was concluded after bordeaux mixture copper hydroxide and onion extract could be potential treatments to control citrus canker on kinnow if their doses and application frequency are optimized. These results are very encouraging as these treatments have no residual effects, hence it will open a door towards organic farming. Further studies are required to evaluate effects of these chemicals to control citrus canker on different citrus varieties

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Contribution of Authors:

Malik A. Rehman	: Designed the experiment and wrote manuscript
Shafqat Ali	: Performed the experiment
Muhammad N. Khan	: Analyzed the data
Salman Ahmad	: Edit the manuscript
Muhammad A. Ali	: Review the manuscript