

INCIDENCE OF CITRUS CANCER DISEASE CAUSED BY *XANTHOMONAS CAMPESTRIS* PV. *CITRI* (HASSE) DOWS ON KINNOW (*CITRUS RETICULATA*) AND ITS CHEMOTHERAPY

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Abstract

Incidence of citrus canker disease revealed 7.5% in three Tehsils Kalurkot, Darya Khan and Bhakkar of the Punjab Province of Pakistan. Among the various toxicants viz., Agrimycin-100, Cupravit, Bavistin, Dithane M-45, Vitavax, Daconil, Antracol, Benlate and Nimrod tested at 1% concentration against multiplication of *Xanthomonas campestris* pv. *citri*. Agrimycin -100, Cupravit, Bavistin, Dithane M-45 and Vitavax proved more effective as compared to other toxicants *In vitro*. All the toxicants @ 1, 0.1 and 0.01% concentrations inhibited the multiplication of the bacterium however, Agrimycin-100 was found to be most effective while Cupravit, Bavistin, Dithane M-45 and Vitavax in that order, were effective against the multiplication of bacterium at 0.01, 0.1 and 1% concentration. Agrimycin-100, Cupravit, Bavistin, Dithane M-45 and Vitavax at 0.2% concentration were sprayed on the field grown citrus plants and then inoculated with *Xanthomonas campestris* pv. *citri* for the control of citrus canker disease. Agrimycin-100, Cupravit, Bavistin, Dithane M-45 and Vitavax in the order proved effective also in reducing the disease intensity as compared to inoculated control.

Introduction

The present day citrus is delectable, juicy, seedless and is of great nutritional significance as well (Khan *et al.*, 1992b). Additionally, it possesses enormous therapeutic qualities (Chaudhry *et al.*, 1992). Although citrus crop is kept in great esteem, yet its present status is threatened by a number of problems, including low production caused by diseases. Citrus plant is attacked by number of diseases like citrus canker, gummosis, citrus decline, CTV, and greening etc. But citrus canker caused by the bacterium *Xanthomonas campestris* pv. *citri*. (Hasse) Dows, is probably the worst enemy to the citrus plantations (Awan *et al.*, 1992). *X.campestris* pv. *citri* is a rod shaped, gram negative bacterium, with single polar flagellum. Growth is obligatory aerobic, maximum temperature for growth is 35-39°C and the optimum temperature is 28-30°C (Mehrotra, 1980; Whiteside *et al.*, 1988).

Controversy still exists on the geographical origin of citrus canker but it is thought to have originated from South – east Asia or India and occurs in more than 30 countries through out the world (Berger, 1914; Civerolo, 1985; Verniere *et al.*, 1998). It is a common and widely distributed disease of Indo-Pak sub continent (Arif *et al.*, 1962). This disease occurs commonly in citrus growing regions of the Punjab that affects leaves, twigs and fruits (Hafiz & Sattar, 1952). Citrus canker is mostly a leaf spotting and rind blemishing disease (Civerolo, 1984).

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The Asiatic form of citrus canker also known as (canker A, cancrrosis A or true canker), caused by *Xanthomonas axonopodis* pv. *citri* (Xac) is a destructive disease that seriously affects most commercially important citrus cultivars grown through out the world. It is one of the biggest problems in citrus production world wide (Stall *et al.*, 1988). *Xanthomonas axonopodis* pv. *citri* has broad host range among members of the Rutaceae, although difference in susceptibility exist in citrus species (Stall & Civerola, 1991). It causes severe symptoms on the grapefruit (*Citrus paradisi* Macf), limes (*C. aurantifolia*, *C. limettioides*), trifoliate orange (*Poncirus trifoliata*) and their hybrids. Symptoms appear as erumpent, callus-like lesions with water soaked, oily, tan colored margins (Verniere *et al.*, 1998). This is considered to be the most widespread and destructive form of the citrus bacterial canker in the world (Koizumi, 1981; Stall Seymour, 1983; Koizumi, 1985; Schoulties *et al.*, 1987., & Gotwald *et al.*, 1993).

In order to manage this disease, resistant stock is the best method but durable host resistance is scarce in local/exotic varieties hence the chemical control is the best alternative to manage citrus canker. The use of chemicals to manage citrus canker has been reported by several research workers. Although antibiotics like Agrimycine-100 and Streptomycine sulphate are the best chemotherapeutant to manage the disease (Leite *et al.*, 1987; Moses & Chandramohan 1993; Masroor, 1995). Application of Streptomycine sulphate and Agrimycin-100 decrease the citrus canker disease (Khan *et al.*, 1992a) but these antibiotics are expensive on one hand and scarcely available on the other hand. Hence it is worthwhile to find out suitable alternative of these antibiotics, which would be cheaper and easily available to the farmers. It would also be useful to find out the longevity of effectiveness of toxicants against the development of disease

Studies were therefore undertaken to find out the incidence of citrus canker in three tehsils of district Bhakkar and to evaluate the efficacy of some available toxicants with different concentrations against *X. campestris* pv *citri*.

Materials and Methods

Survey of citrus canker incidence: In order to record the incidence of citrus canker, survey was conducted in citrus orchard of Kinnow (*Citrus reticulata*) at different localities of Kalurkot, Daraya Khan and Bhakkar tehsils of the Punjab province. Five citrus orchards in each of the above mentioned localities were randomly selected. From each orchard 10 Kinnow plants were selected randomly and the disease intensity was recorded according to the disease rating scale described by Horsfall & Heuberger, (1942).

0 = Free from infection

1 = Traces to 25% leaf area killed

2 = 26-50% leaf area killed

3 = 51-75% leaf area killed

4 = 76-100% leaf area killed

An infection index was then obtained by the following formula:

$$\text{Infection Index} = \frac{\text{Sum of individual rating}}{\text{Number of plants assessed}} \times \frac{100}{4}$$

In vitro evaluation of various toxicants against *Xanthomonas campestris* pv. *citri*: Sensitivity of *X. campestris* pv. *citri*, to various toxicants was studied by using techniques described by Cruickshank *et al.*, (1975). Filter paper discs 1 cm diameter were cut with

the help of cork borer and sterilized in an autoclave at 1.1 Kg/cm² for 15 minutes. These discs were then impregnated with 1% solution of Agrimycin-100, Cupravit, Bavistin, Dithane M-45, Vitavax, Daconil, Antracol, Benlate, Nimrod, and Afugan. Bacterial suspension of *X. campestris* pv. *citri* (approx. 10⁸ CFU/ml) was prepared. One milliliter of this suspension was poured in sterilized Petri dishes on to which about 20 ml of sterilized luke warm nutrient agar was poured. The Petri dishes were gently shaken to mix the bacterial cell suspension uniformly on the nutrient agar. The mixture was then allowed to solidify.

For each set of toxicant, impregnated discs were then placed 3 cm apart on the solidified nutrient agar containing the bacterium in Petri dishes. These Petri dishes were then incubated at 30°C for 48 hours, and inhibition zones, around the discs if any were recorded as described by Buxton & Fraser (1977). Experiment was conducted with three replication having four Petri dishes/replication. Control was similarly included with discs dipped in sterilized water. Data recorded on the inhibition zones were statistically analyzed by using DMR test for the comparison of means (Steel *et al.*, 1996).

***In vitro* efficacy of different concentrations of toxicants against *Xanthomonas campestris* pv. *citri*:** Relatively more effective toxicants from the previous experiment were further tested at 1, 0.1 and 0.01% concentrations against *X. campestris* pv. *citri*. Sterilized petri plates containing one milliliter suspension (having 10⁸ CFU/ml) of *X. campestris* pv. *citri*, were poured with luke warm nutrient agar. The Petri plates were gently shaken to mix the bacterial suspension with nutrient agar and placed them to solidify. Ten mm (1cm) diameter autoclaved filter paper discs were dipped in each of the three concentrations (1, 0.1 and 0.01%) of Agrimycin-100, Cupravit, Bavistin, Dithane M-45 and Vitavax. The toxicant impregnated discs were placed in the bacterial mixed agar plates. Petri plates were then incubated at 30°C for 48 hours. The Petri plates in the control treatment had filter paper discs dipped only in sterilized water. All the treatments were triplicates (three Petri plates/ replications). The data on the zone of inhibition of *X. campestris* pv. *citri* around the discs for each treatment were recorded, and statistically analyzed (Steel *et al.*, 1996).

Field evaluation of various toxicants against *Xanthomonas campestris* pv. *citri*: One year old, healthy citrus plants variety kinnow (*C. reticulata*) were sprayed with Agrimycin-100, Cupravit, Bavistin, Dithane M-45 and Vitavax at 0.2 %. After 24 hours of treatment, the plants were irrigated and covered with polyethylene bags for about two hours to promote maximum humidity, followed by inoculation with *Xanthomonas campestris* pv. *citri* suspension with the help of spray machine with a pressure of 1.1 Kg cm⁻². The plants inoculated with distilled sterilized water only served as control. The data regarding disease intensity were recorded at 5 days interval up to 45 days after inoculation, (Croxall *et al.*, 1952).

Results

Citrus canker incidence in Bhakkar district: Survey conducted in three tehsils revealed that the incidence of citrus canker disease was 7.5%. Citrus orchard situated at tehsil Kalurkot showed 7.5, 7.5, 7.6, 7.8 and 7.5% disease incidence in different localities while data recorded on the disease severity in the citrus orchard in tehsil Darya Khan showed 7.8, 7.5, 8.0, 7.5 and 7.5% citrus canker infection in different localities (Table 1). In case of tehsil Bhakkar the % incidence of infection varied from 7.5, 7.1, 7.5, 8.0 and 7.6% at different localities.

Table 1. Incidence of citrus canker in different Tehsils of district Bhakkar, Punjab, Pakistan.

S. No.	Location	Percent Disease Average of randomly selected 10 plants in each orchard			
		1	2	3	Mean
Tehsil Kallurkot					
1.	Government Faridia Garden	7.5	8.0	7.0	7.5
2.	Abbasianwala	8.0	7.5	7.0	7.5
3.	Saeedwala	6.5	8.0	8.5	7.6
4.	Chak # 65 D.B (Jandawala)	7.5	9.0	7.0	7.8
5.	Chak # 33 R.D (Dulewala)	8.5	7.5	6.5	7.5
Tehsil Darayakhan					
1.	Chak.No.14 T.D.A. Skiandarabad	7.5	6.5	8.0	7.8
2.	Lot. No.31 (Barkatwala)	8.0	7.5	7.0	7.5
3.	Chak. No. 9 T.D.A.	8.5	8.0	7.5	8.0
4.	Chak No. 6 T.D.A.	8.0	7.5	7.0	7.5
5.	Chak No.46T.D.A.	7.0	8.0	7.5	7.5
Tehsil Bhakkar					
1.	Chak .No 50 T.D.A. (Khanwala)	8.0	7.5	7.0	7.5
2.	Chak No.175 T.D.A. (Sarai Mahajar)	8.5	7.0	6.0	7.1
3.	Chak No. 83 T.D.A. (Chattiwala)	8.0	7.5	7.0	7.5
4.	Kotla Jam	7.0	8.0	7.5	8.0
5.	Chak No. 27 T.D.A.	9.0	7.5	6.5	7.6

Table 2. Comparison of means of different toxicants at 1 % concentration against *Xanthomonas campestris* pv. *citri* In vitro (Inhibition zones (cm) after 48 hours).

S. No.	Toxicants	Mean	Percent decrease over control
1.	Agrimycin-100	2.89 a	32.1
2.	Cupravit	2.46 b	27.3
3.	Bavistin	2.35 c	26.1
4.	Dithane M-45	2.00 c	22.2
5.	Vitavax	1.80 c	20.0
6.	Daconil	1.53 d	17.0
7.	Antracol	1.43 d	16.0
8.	Benlate	1.00 e	11.1
9.	Nimrod	0.60 f	9.0
10.	Afugan	0.00 g	0.00
11.	Control	0.00 g	0.00

Means sharing same alphabets are statistically non-significant at p=0.05 (DMR Test)

In vitro evaluation of various toxicants against *Xanthomonas campestris* pv. *citri*: All the toxicants reduced the multiplication of *X. campestris* pv. *citri* significantly as compared to control but they varied greatly in their effect (Table 2). Agrimycin-100, Cupravit, Bavistin, Dithane M-45 and Vitavax @ 1% concentration were found to be the most effective toxicants in inhibiting the growth of the bacterial culture as inhibition zones recorded in these toxicants were 2.89, 2.46, 2.35 and 2.0 and 1.80 cm, respectively.

The other toxicant viz., Daconil, Antracol, Benlate, Nimrod, and Afugan at 1% concentration were comparatively less effective in inhibiting the bacterial growth as indicated by 1.53, 1.43, 1.00 0.60 and 0.00 cm inhibition zones for each fungicide.

Efficacy of different concentrations of toxicants against *Xanthomonas campestris* pv. *citri* in vitro. Agrimycin-100, Cupravit, Bavistin, Dithane M-45, and Vitavax which proved effective at 1% concentration against the multiplication of bacterium, were further tested at 1, 0.1 and 0.01% concentrations. Data recorded on the inhibition zones revealed that all the toxicants at all the concentrations reduced bacterial growth significantly compared with control. However, there was an increase in inhibition zone with an increase in concentration of toxicants. Agrimycin-100, Cupravit at 0.1% concentration, in that order, were found to be the most effective toxicants in inhibiting the growth of the bacterial culture as the inhibition zones diameter recorded in these cases were 2.78 and 2.51 cm, respectively. On the other hand, Bavistin, Dithane M-45 and Vitavax at 0.1% concentration were comparatively less effective in inhibiting the bacterial growth as indicated by 2.15, 1.45 and 1.38 cm inhibition zones. Agrimycin-100 and Cupravit at 0.01% concentration inhibited the bacterial growth more effectively as the inhibition zones recorded in these toxicants were 2.35 and 1.98 cm, respectively, while Bavistin, Dithane M-45 and Vitavax at 0.01% concentration proved less effective than Agrimycin-100 and Cupravit as the inhibition zones recorded for these toxicants were 1.75, 1.32 and 0.98 cm, (Table 3).

Field evaluation of various toxicants against *Xanthomonas campestris* pv. *citri*. None of the toxicants used completely inhibited the symptom development however the intensity of disease was decreased significantly than the inoculated control. The disease intensity increased progressively with the passage of time. Plants sprayed with Agrimycin-100, Cupravit, Bavistin, Dithane M-45 and Vitavax, each at 0.2% concentration and then inoculated with *X. campestris* pv. *citri* exhibited infection index values of 1.93, 2.20, 2.30, 2.50 and 2.70, as compared with 2.83 in case of control, 10 days after inoculation. Infection index values recorded 15 days after inoculation on plants were 2.07, 2.57, 2.50, 2.63 and 2.83 by Agrimycin-100, Cupravit, Bavistin, Dithane M-45, and Vitavax, however, the value of infection index was 2.97 in case of control. The values of infection index recorded in plants were 2.37, 2.67, 2.67, 2.73 and 3.03 on Agrimycin-100, Cupravit, Bavistin, Dithane M-45 and Vitavax, treated plants while in case of control 3.17 infection index was recorded 20 days after inoculation, plants sprayed with Agrimycin-100, Cupravit, Bavistin, Dithane M-45, and Vitavax, exhibited an infection index values of 2.53, 2.80, 2.83, 2.83 and 3.33 after 25 days of inoculation, while in case of control an infection index value of 3.40 was recorded. Thirty days after inoculation, the infection index value recorded in case of Agrimycin-100, Cupravit, Bavistin, Dithane M-45 and Vitavax, sprayed plants were 2.70, 2.90, 3.07, 2.93 and 3.47, as compared with 3.67 in case of control 30 days after inoculation (Table 4). The values of infection index recorded in plants were 2.77, 3.07, 3.32, 3.27 and 3.73 by Agrimycin-100, Cupravit, Bavistin, Dithane M-45 and Vitavax, while in case of control 4.10 infection index was recorded 35 days after inoculation. Plants sprayed with Agrimycin-100, Cupravit, Bavistin, Dithane M-45 and Vitavax exhibited an infection index values of 2.90, 3.23, 3.40, 3.57 and 4.00 after 40 days of inoculation, while in case of control an infection index value of 4.43 was recorded. Forty five days after inoculation, the infection index value recorded in case of Agrimycin-100, Cupravit, Bavistin, Dithane M-45, and Vitavax sprayed plants were 3.03, 3.37, 3.60, 3.87 and 4.20 as compared with 4.83 in case of control (Table 4).

Table 3. Comparison of efficacy of different concentrations of toxicants against *Xanthomonas campestris* pv. *citri* In vitro.

S. No.	Toxicants	Concentrations %			Mean
		1	0.1	0.01	
1.	Agrimycin-100	2.89 a	2.78 a	2.35 bc	2.67 a
2.	Cupravat	2.46 b	2.51 b	1.98 def	2.32 b
3.	Bavistin	2.35 bc	2.15 cd	1.75 f	2.08 c
4.	Dithan M-45	2.00 de	1.45 g	1.32 g	1.59 d
5.	Vitavax	1.80 ef	1.38 g	0.98 h	1.39 e
6.	Control	0.00 i	0.00 i	0.00 i	0.00 f
	Mean	1.92 a	1.71 b	1.39 c	

LSD value 0.04833

Means sharing same alphabets are statistically non significant at P=0.05 (DMR Test)

Table 4. Evaluation of various toxicants against *Xanthomonas campestris* pv. *citri* at 0.2% concentration.

Toxicants	Days after inoculation								Mean
	10	15	20	25	30	35	40	45	
Agrimycin-100	1.93 u	2.07 tu	2.37 q-u	2.53 o-u	2.70 k-t	2.77 j-t	2.90 i-s	3.03 g-q	2.54 e
Cupravat	2.20 s-u	2.57 n-u	2.67 l-t	2.80 j-s	2.90 i-s	3.07 g-q	3.23 f-o	3.37 e-l	2.85 d
Bavistin	2.30 r-u	2.50 p-u	2.67 l-t	2.83 j-s	3.07 g-q	3.32 f-o	3.40 d-k	3.60 c-i	2.95 cd
Dithan M-45	2.50 p-u	2.63 m-u	2.73 k-t	2.83 j-s	2.93 i-r	3.27 f-n	3.57 f-n	3.87 b-f	3.04 c
Vitavax	2.70 k-t	2.83 j-s	3.03 g-q	3.33 e-m	3.47 d-j	3.73 b-g	4.00 b-e	4.20 a-c	3.41 b
Control	2.83 j-s	2.97 h-r	3.17 f-p	3.40 d-k	3.67 c-h	4.10 b-d	4.43 a-b	4.83 a	3.68 a
Mean	2.41 g	2.59 fg	2.77 ef	2.96 de	3.12 d	3.36 c	3.59 b	3.82 a	0.2439

Mean sharing the same alphabets are statistically non significant at p=0.05 (DMR Test)

Discussion

Asiatic citrus canker induced by *X. axonopodis* pv. *citri* has re-emerged as potential threat to citrus plantation throughout the world (Gottwald *et al.*, 2001). The citrus cultivars previously known to be resistant to this pathogen have now become susceptible. Once this disease becomes endemic in an area, it is very difficult to manage with commercially acceptable methods under favorable conditions for disease development (Das, 2003). In the present studies emphasis was to conduct survey in different tehsils of district Bhakkar to ascertain the occurrence/ incidence of citrus canker on Kinnow (*Citrus reticulata*) cultivar and evaluation of different toxicants for its management. The incidence of citrus canker varied from 7-7.5% in different localities in three tehsils of district Bhakkar. This might be due to some factors including environmental conditions prevailing during the month of January-February. Similar results have been reported by Khan *et al.*, (1992a) who recorded 10-12.5% incidence of citrus canker caused by *Xanthomonas campestris* pv. *citri* in three tehsils of Faisalabad district. As far as the management of citrus canker is concerned, the most effective mean is by supplementing the use of resistant cultivars with integrated system of compatible cultural practices and phytosanitary measures, including quarantine and regulatory programmes (Das, 2003). One component of integrated disease management is the use of chemicals for bacterial plant pathogens. Worldwide use of copper based bactericides is considered to be the standard control measure for citrus canker (Koizumi, 1985; Leite & Mohan, 1990). Copper application as multiple doses reduced the bacterial population on the leaf surfaces on the susceptible host (Stall *et al.*, 1980). Effective suppression of the disease by copper sprays depends on several factors, such as the susceptibility of the citrus

cultivar, environmental conditions and adoption of other control measures (Kuhara, 1978, Stall & Seymour, 1983; Leite & Mohan., (1990). In the present experiments the toxicants varied greatly for their affect on the inhibition of *X. campestris* pv. *citri* *in vitro* and there was an increase in the zones of inhibition with an increase in the concentration of toxicants. The effect of Agrimycin-100 was much pronounced as compared with other chemicals. Agrimycin-100 at 0.01, 0.1 and 1% concentration was found to be the most effective toxicant while Cuparvit, Bavistin, Dithane M-45 and the Vitavax in that order proved significantly effective in inhibiting the growth of the bacterium. The effectiveness of Agrimycin-100 against *X. campestris* pv. *citri* has been reported by various research workers (Rangasawami *et al.*, 1959; Nirvan, 1960; Balaraman *et al.*, 1981; Sawant *et al.*, 1985 & Sothosorumbini *et al.*, 1986). Similarly effectiveness of Dithane M-45 against *X. campestris* pv. *citri* for the control of citrus canker has been reported by Liu, (1966) who observed that spraying of Dithane M-45 + Copper sulphate, either before or after inoculation gave good control of citrus canker. The other toxicants like Cupravat and Bavistin at 1% concentration displayed equal level of effectiveness but Cupravat was more effective than Bavistin. Agrimycin-100, Cupravat, Bavistin, Dithane M-45 and Vitavax at 0.2% concentration sprayed on citrus plants as protectants inhibited the symptoms development produced by artificial inoculation with *Xanthomonas campestris* pv. *citri*. The inhibiting effect of these toxicants remained apparent uptill 10 days after inoculation, when the symptoms of citrus canker disease started appearing on the citrus plants. The results showed that Agrimycine-100 proved to be the best toxicant and the disease intensity increased with the passage of time under the field condition. Agrimycine-100 @ of 1000 ppm has been reported to give promising results in controlling the citrus canker disease (Leite *et al.*, 1987 & El-Goorani 1989), where as streptomycin sulphate @ 500 ppm with four spray schedules significantly reduce the disease intensity (Chakarvarti *et al.*, 1970 ; Vibhute *et al.*, 1975). Similar findings culminating into good control of citrus canker were reported by Krishna & Nema (1983) where application of antibiotics as well as the fungicides was found effective but the better control was achieved by the application of antibiotics. Antibiotics in combination with fungicides can effectively reduce the disease incidence. Such bactericidal activity of some fungicides has been reported by Khan *et al.*, (1992a), Akhtar *et al.*, (1996) who indicated that Streptomycin sulphate, Agrimycin-100, Vitavax, Dithane M-45 and Ridomil were the most effective as antibacterial toxicants.

Regarding the chemical control of the pathogen some of available toxicants tested against the bacterium although very effective *In vitro* but failed to eradicate the pathogen completely in the field evaluation. This may due to systemic nature of the pathogen for which several sprays of the toxicants are recommended and in this case benefit cost ratio factor plays an important role.

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