INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2005/07–1–121–124
http://www.ijab.org

Evaluation of Different Toxicants Against *Xanthomonas campestris pv. citri* (Hasse) Dows

SHAHID M. MAHER, S.T. SAHI, M. USMAN GHAZANFAR, M. INAM-UL-HAQ, IMRAN-UL-HAQ, YASIR IFTIKHAR, M.S. SARWAR AND TAUQIR AHMAD
Department of Plant Pathology, University of Agriculture, Faisalabad–38040, Pakistan

ABSTRACT

Toxicants (Streptomycin sulphate Dithane M-45, Agrimycin – 100, Vitavax, Benlate and Cobox) were tested at 1% concentration against multiplication of *Xanthomonas campestris* pv. *citri in vitro*. Agrimycin –100, Streptomycin sulphate, Vitavax, and Dithane M – 45 proved more effective as compared to other toxicants. Agrimycin –100, Streptomycin sulphate DithaneM-45 and Vitavax were further studied *in vitro* against the growth of *Xanthomonas campestris* pv. *citri* at 0.01 , 0.1 and 1% concentration. All the toxicants inhibited the multiplication of the bacterium at all concentrations, however Streptomycin sulphate was found to be the most effective among the toxicants used while Agrimycin –100, Vitavax and Dithane M –45, in the order, were effective against the multiplication of bacterium at 0.01, 0.1 and 1% concentration. The inhibition zone recorded in the toxicants was increased. Streptomycin sulphate, Agrimycin – 100, Vitavax, Dithane M –45 and Benlate at 0.2% concentration were sprayed on the field grown citrus plants and then inoculated with *Xanthomonas campestris* pv. *citri* for control of citrus canker disease. Streptomycin sulphate, Vitavax, Dithane M –45 and Agrimycin –100 in the order proved effective also in reducing the disease intensity as compared to inoculated control.

Key Words: Toxicants; *Xanthomonas campestris* pv. *citri*

INTRODUCTION

Citrus belongs to family Rutaceae and Sub family Aurantiodeae. Botanically, all commonly cultivated citrus fruits are classed under three genera, i.e. Citrus, Fortunella and Poncirus (Braverman, 1949). Citrus has got high nutritive and refreshing value and is considered to be the best source of Vitamin C, Sugars, Amino acids and other nutrients (Ahmed & Khan, 1999). The present day citrus is delectable, juicy, seedless, and is of great nutritional significance as well (Khan, 1992a). Additionally, it possesses enormous therapeutic qualities (Chaudhry, 1992).

Canker apparently originated in South East Asia but it was first observed in Florida in 1912 (Berger, 1914). 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, 1964).

In Pakistan, average production of citrus fruit is 9.5 tones per hectare only (Walter, 1989), and it is only because citrus industry faces many disorders / factors which impede the fruit yield and quality. Diseases are one of the major factors which may affect the plant health and fruit development adversely. 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).

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 (Leite *et al.*, 1987; Moses & Chandramohan 1993; Masroor, 1995).

Therefore, the studies were carried out to evaluate the efficacy of some toxicants with different concentrations against *X. campestris* pv *citri*.

MATERIALS AND METHODS

***In vitro* evaluation of various toxicants against *Xanthomonas compestris* 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/cm^2 for 15 min. These discs were then impregnated with 1% solution of Streptomycin sulphate, Agrimycin – 100, Vitavax, Dithane M – 45, Ridomil, Antracol, Pencozeb, Liromanzeb, Polyramcombi, Cobox and Benlate. Bacterial suspension (approx. 10^8 cells mL^{-1}) of *X. campestris* pv. *citri* was

prepared as described previously. 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 bacteria uniformly in the nutrient agar. The mixture was then allowed to solidify.

Each set of toxicant, impregnated discs were then placed 3 cm apart on the solidified nutrient agar containing the bacterium in petri dishes. These were then incubated at 30°C for 48 h, and inhibition zones, around the discs if any were recorded as described by Buxton and 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).

Efficacy of different concentrations of Toxicants against *Xanthomonas campestris pv. citri in vitro.* 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 10^8 cells/mL) of *X. campestris* pv. *citri*, were poured with luke warm nutrient agar. The petriplates were gently shaken to mix the bacterial suspension with nutrient agar and place them to solidify. 10 mm (1cm) diameter autoclaved filter paper discs were dipped in each of the three concentrations (1, 0.1 and 0.01%) of Streptomycin sulphate, Agrimycin –100, Vitavax, Dithane M –45 and Ridomil. The toxicant impregnated discs were placed in the bacterial mixed agar plates. Petriplates were then incubated at 30°C for 48 h. The petriplates in the control treatment had filter paper discs dipped only in sterilized water. All the treatments were triplicated (three petriplates / replications). The data on the zone of inhibition of *X. campestris* pv. *citri* around the discs for each treatment were recorded, and statistically analyzed by using Bartletts method for the comparison of means as described by (Steel *et al*., 1996).

Evaluation of various toxicants against *xanthomonas campestris* pv. *citri.* One year old, healthy citrus plants were sprayed with Streptomycin sulphate, Agrimycin –100, Vitavax, Dithane M –45 and Benlate at 0.2%. After 24 h 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 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 five days interval upto 30 days after inoculation following Croxall *et al.* (1952).

RESULTS

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 I). Agrimycin – 100, Streptomycin sulphate, Vitavax, Dithane M-45 at 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 2.47, 2.28, 2.38 and 2.32 cm, respectively.

The other toxicants Benlate and Cobox at 1% concentration were comparatively less effective in inhibiting the bacterial growth as indicated by 0.68, and 0.47 cm inhibition zones for each fungicide respectively.

Table I. Comparison of Means of different toxicants at (1 percent concentration) against *Xanthomonas campestris Pv. citri* in vitro (inhibition zones (cm) after 48 hours)

	Toxicants	Mean
1.	Agrimycin-100	2.47 A
2.	Benlate	0.68 B
3.	Cobox	0.47 BC
4.	Dithane M-45	2.32 A
5.	Vitavax	2.38 A
6.	Streptomycin sulphate	2.28 A
7.	Control	0.00 C

Means sharing same alphabet are statistically non-significant

Efficacy of different concentrations of toxicants against *Xanthomonas campestris* pv. *citri in vitro.* Agrimycin –100, Dithane M-45, Streptomycin sulphate 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, Streptomycin sulphate, 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 2.88 cm, respectively. However, Dithane M-45 and Vitavax at 0.1% concentration were comparatively less effective in inhibiting the bacterial growth as indicated by 1.80 and 2.37 cm inhibition zones, respectively. Streptomycin sulphate, Agrimycin –100 at 0.01% concentration inhibited the bacterial growth more effectively as the inhibition zones recorded in these toxicants were 2.02 and 2.04 cm, respectively.

While Dithane M-45 and Vitavax at 0.01% concentration proved less effective than Streptomycin sulphate, and Agrimycin –100 as the inhibition zones recorded for these toxicants were 1.17 and 1.70 cm, respectively (Table II).

Table II. Comparison of efficacy of different concentrations of toxicants against *Xanthomonas campestris Pv. citri* in vitro

Toxicants	Concentrations			Mean
	1%	0.1%	0.01%	
Agrimycin-100	2.78 ab	2.70 b	2.04 e	2.51 A
Dithane M-45	1.80 f	1.40 g	1.17 h	1.46 C
Streptomycin sulphate	2.88 a	2.80 ab	2.02 e	2.57 A
Vitavax	2.37 c	2.17 d	1.70 f	2.08 B
Control	0.00 I	0.00 I	0.00 I	0.00 D
Mean	1.97 A	1.81 B	1.38 C	0.04833

LSD value 0.04833
Means sharing same alphabet are statistically non significant

Evaluation of various toxicants against *Xanthomonas campestris* pv. *citri.* None of the toxicants used (Streptomycin sulphate, Agrimycin –100) completely inhibited the symptoms development however the intensity of disease was decreased significantly than the inoculated control. The disease intensity increased progressively with passage of time. Plants sprayed with Streptomycin sulphate, Agrimycin –100, Vitavax, Dithane M-45 and Benlate each at 0.2% concentration and then inoculated with *X. campestris* pv. *citri* exhibited infection index values of 3.00, 2.33, 2.40, 1.90 and 1.83, respectively as compared with 2.93 in case of control, 10 days after inoculation. Infection index values recorded 15 days after inoculation on plants were 3.20, 2.53, 2.60, 2.13 and 2.07 by Streptomycin sulphate, Agrimycin 100, Vitavax, Dithane M-45 and Benlate, respectively; however, the value of infection index was 3.13 in case of control. The values of infection index recorded in plants were 3.40, 2.67, 2.73, 2.33 and 2.27 Streptomycin sulphate, Agrimycin –100, Vitavax, Dithane M-45 and Benlate, respectively while in case of control 2.27 infection index was recorded 20 days after inoculation, plants sprayed with Streptomycin sulphate, Agrimycin –100, Vitavax, Dithane M-45 and Benlate exhibited an infection index values of 3.53, 2.47, 2.87, 2.53 and 2.47, respectively after 25 days of inoculation, while in case of control an infection index value of 3.50 was recorded. 30 days after inoculation, the infection index value recorded in case of Streptomycin sulphate, Agrimycin –100, Vitavax, Dithane M-45 and Benlate sprayed plants were 3.67, 2.93, 3.07, 2.73 and 2.60, respectively as compared with 4.17 in case of control (Table III). So, the Streptomycin Sulphate, Vitavax, Agrimycin-100, Dithane M-45, in that order proved effective against citrus canker disease.

Table III. Evaluation of various toxicants against *Xanthomonas campestris Pv. citri* (at 0.2% concentration)

Toxicants	Days after inoculation					Mean
	10	15	20	25	30	
T1	1.83	2.07	2.27	2.47	2.60	2.25 C
T2	2.33	2.53	2.67	2.73	2.93	2.64 B
T3	3.00	3.20	3.40	3.53	3.67	3.36 A
T4	1.90	2.13	2.33	2.53	2.73	2.33 C
T5	2.40	2.60	2.73	2.87	3.07	2.73 B
T6	2.93	3.13	2.27	3.50	4.17	3.40 A
Mean	2.40	2.61	2.78	2.94	3.19	0.2439
	D	CD	BC	B	A	

Means sharing same alphabet are statistically non significant

DISCUSSION

The toxicants varied greatly for their affect on the inhibition of culture of *X. campestris* pv. *citri* and there was an increase in the zones of inhibition with an increase in the concentration of toxicants solution into which discs were dipped.

Streptomycin sulphate at 0.1 and 1% concentration was found to be the most effective toxicant while Agrimycin –100 was found to be most effective at 0.01%. While Vitavax, and Dithane M-45 in that order proved less effective than Streptomycin sulphate and Agrimycin –100 in the inhibition of the bacterial culture. However, Benlate and Cobox at 1% concentration displayed very little effectiveness. Fungicides are generally poor bactericides, but Vitavax proved to be the almost as effective as the antibiotic Agrimycin –100. Streptomycin sulphate, Agrimycin –100 Vitavax, Dithane M-45 and Benlate at 0.2% concentration sprayed on citrus plants as protectant inhibited the symptom development produced by artificial inoculation with *X. campestris* pv. *citri.* The inhibiting effect of these toxicants was apparent 10 days after inoculation when the symptoms of citrus canker disease appeared on the citrus plants.

The effect of Streptomycin Sulphate was much pronounced as compared to other chemical The effectiveness of Streptomycin Sulphate against *X. campestris* pv. *citri* for the control of citrus canker has been reported by various research workers (Rangasawami *et al.,* 1959; Nirvan, 1960; Balaraman *et al.,* 1981; Sothosorumbini *et al.,* 1986). However, Leite *et al.* (1987) and El-Goorani (1989) reported the ineffectiveness of Streptomycin sulphate against citrus canker effectiveness of Dithane M – 45 against *X. campestris* pv. *citri* for the control of citrus canker has been reported by various research workers. Liu (1966) reported that spraying of Dithane M – 45 + Copper Sulphate, either before or after inoculation gave good control of citrus canker. Chakarvarti *et al.* (1970) and Vibhute *et al.* (1975) achieved best control of citrus canker with Agrimycin –100 at the rate of 1000

ppm concentration and Krishna and Nema (1983) reported that best control was achieved with Streptomycin at 500 ppm with four sprays schedule. Khan *et al*. (1992) reported that Streptomycin sulphate, Agrimycin-100, Vitavax, Dithane M-45 and Ridomil were the most effective as antibacterial. Akhtar *et al.* (1996) reported that Streptomycin Sulphate, exhibited inhibitory effect on all strains. Jadeja *et al*. (2000) reported that canker control was achieved with foliar application of Streptomycin sulphate + Copper oxychloride.

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. 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.

REFERENCES

Ahmed, R. and H.U. Khan, 1999. Citrus decline: problems and progress in the Punjab, pp: 20–2. A Review: *Proc. 2nd Nat. Conf. Pl. Path.* University of Agriculture, Faisalabad–Pakistan

Akhtar M.A., M.H.R. Bhatti and M. Aslam, 1996. Effect of toxicants on *X. campestris* pv. *citri*. Strains. *Pakistan J. Phytopathol*., 8: 113–8

Arif, A.G., M. Akhtar and M. Ibrahim, 1962. Citrus diseases and their control. *Punjab Fruit J*., 26/27: 369–70

Awan. M.Z., M. Ishfaq, I.A. Hafiz, M. Ijaz, and C.M. Ayyub, 1992. Incidence of citrus canker in Barani area at Chakwal. pp: 315–8. *Proc. 1st Int. Sem. Citriculture in Pakistan*, Dec.2–5. University of Agriculture Faisalabad–Pakistan

Balaraman, K. and R. Purusotman, 1981. Control of citrus canker on acid lime. Indian Institute of Horticultural Research, Bangalore, India. *South Indian Horticulture*, 29: 175–7

Berger, E.W., 1914. Citrus canker. II. History of citrus canker in Florida. *Agric. Exp. Sta. Bull*., 124: 27–30

Braverman, J.B.S., 1949. *Citrus products: Chemical Composition and Chemical Technology*, p: 5–7. Inter–Science Publishers Inc. New York

Buxton, A. and G. Fraser, 1977. *Animal Microbiology*, Vol. I. Blackwell Scientific Publication, Oxford London, Edinburg Melbourne

Chakravarti. B.P. and S.V. Hedge, 1970. Studies on citrus canker in Rajasthan III. Control of citrus canker by antibiotics and chemicals. *Punjab Hort. J*., 19: 161–5

Chaudhry, N.A., A.R. Khan and Hameedullah, 1992. *Introduction of acclimatized exotic citrus*. p: 15. Citrus fruit varieties at Horticultural Research Station, Sahiwal. *Proc. 1st Int. Sem. Citriculture in Pakistan*, Dec.2–5. University of Agriculture Faisalabad–Pakistan

Civerolo, E.L., 1964. Bacterial canker disease of citrus. *J. Rio. Grande Val. Hortic. Soc*., 37: 127–46

Croxall, H.E., D.C. Gwynne and J.E.E. Jenkins. 1952. The rapid assessment of apple scab on leaves. *Pl. Pathol.*, 1: 39–41

Cruickshank, R., J.P. Dugind, B.P. Marmion and R.H.A. Swain, 1975. Tests for Sensitivity to Antibacterial Agents. pp: 190–208. Medical Microbiology 12th Ed., Vol. II

El–Goorani, M.A., 1989. The occurrence of citrus canker disease in United Arab Emirates (U.A.E). *J. Phytopath*, 123: 257–64

Hafiz, A. and A. Sattar, 1952. *Canker of Citrus: Research on Plant Diseases of the Punjab*, pp: 142–5. Pakistan Association for the development of Science, Lahore –Pakistan

Khan, I.A., 1992a. Key note, Prospects of the citrus industry in Pakistan, pp: 3–10. *Proc. 1st Int. Sem. Citriculture in Pakistan*, 2–5 Dec. University of Agriculture Faisalabad–Pakistan

Khan, I.A., M.J. Jaskani and S.N.H. Ali, 1992. *Breeding for Seedless Kinnow, a Progress Report*. pp: 103–55. *Proc. 1st Int. Sem. Citriculture in Pakistan*, Dec.2–5. University of Agriculture Faisalabad–Pakistan

Krishna, A. and A.G. Nema, 1983. Evaluation of chemicals for the control of citrus canker. *Indian Phytopath*., 36: 348–50

Leite–Junior, R.P., S.K. Mohan, A.G. Pereira and C.A. Compacci, 1987. Integrated control of citrus canker effect of genetic resistance and application for bactericides. *Phytopathologia–Braseleira*, 13: 257–63

Leite–Junior, R.P., S.K. Mohan, A.G. Pereira and C.A. Compacci, 1987. Integrated control of citrus canker effect of genetic resistance and application for bactericides. *Phytopathologia–Braseleira*, 13: 257–63

Liu, K.C., 1966. Studies on the control of citrus canker. *J. Taiwan Agric. Res*., 15: 49–52

Masroor, M.K. and S. Chaudra, 1995. Effect of temperature on antibiotic production by *Aspergillus spp*. Antagonistic of citrus canker pathogen. *Bioved*., 6: 65–8

Mehrotra. R.S., 1980. *Plant Pathology*, pp: 636–8. Tata McGraw–Hill pub. Co. Ltd. New Delhi

Moses, G.J. and B. Chandramohan, 1993. Potential of Neem and other plant extracts for management of crop diseases. *In:* Mariappan, V. (ed.) Neem for the management of crop diseases, pp: 91–7. Associated Pub. Co., New Delhi

Nirvan, R.S., 1960. Effect of antibiotics spray on citrus canker. *Hort. Advances*, 4: 155–60

Rangaswami, G., R.R. Rao and A.R. Lakshamanan, 1959. Studies on the control of citrus canker with Streptomycin. *Phytopath*., 49: 224–6

Sothiosorubini, N., R.S.V. Sundersan and A. Sivapalan, 1986. Studies on *Xanthomonas pv. citri*, causing canker disease of citrus. *Vingnanam J. Sci.*, 1: 19–25

Steel, R.G.D, T.H. Torrie and D. Dickey, 1996. *Principles and Procedures of Statistics. A Biometrical Approach*, 3rd Ed. McGraw Hill Book Co., New York, USA

Vibhute, Y.B. and S.S. Wadge, 1975. Antimicrobial activity of some halogene – flavones and flavanones. *Hindustan Antibiotics Bull*., India. 18(1/2): 42–4

Walter J.K., 1989. *Citrus research in Pakistan: Problems and Solutions*, p: 44. A Consultant Report, PARC. USA. Mart. WinRock

Whiteside, J.O., S.M. Garney and L.W. Timmer (eds), 1988. Compendium of citrus diseases. *The Amer. Phytopath. Soc*., p: 80

(Received 12 October 2003; Accepted 18 December 2004)