Pak. J. Bot., 35(5): 967-970, 2003.

IN-VITRO EVALUATION OF PLANT EXTRACTS AND ANTAGONISTIC FUNGI AGAINST *XANTHOMONAS CAMPESTRIS* PV. *CITRI* (HASSE) DYE

F. IQRAM, M. A. KHAN AND S. M. KHAN

Department of Plant Pathology,
University of Agriculture, Faisalabad, Pakistan.

Abstract

Sensitivity of *Xanthomonas campestris* pv. *citri* was tested by dual culture plate assays using 10 plant extracts *viz. Acacia modesta* (Phulai), *Acacia nilotica* (Babool), *Allium sativum* (Garlic), *Bougainvillea spectabilis* (Bougainvillea), *Dalbergia sisso* (Shisham), *Datura alba* (Dhatura), *Eucalyptus camelduleusis* (Sufeda), *Ficus religiosa* (Pipal), *Olea europaea* (Olive) and *Phyllanthus emblic* (Emblic) and six antagonistic fungi *viz. Aspergillus flavus, Aspergillus niger, Baeuveria bassiana, Paecilomyces lilacinus, Trichoderma harzianum* and *Verticillium chlamydosporium*. Except *Ficus religiosa*, all the plant extracts at standard concentration (75 g fresh leaves + 25 mL sterilized water) reduced the multiplication of *X. camp.* pv. *citri* significantly compared to control. *Allium sativum* was the best followed by *A. nilotica, D. alba, D. sisso* and *A. modesta* inducing 43, 24, 24, 23.67 and 17.78 % inhibition of the bacterial growth over control, respectively. *Phyllanthus emblica, E. camelduleusis* and *B. spectabilis* were statistically at par in their effectiveness. All the antagonistic fungi significantly reduced the bacterial multiplication than control. *Aspergillus flavus* was the best antagonist, while *A. niger, T. harzianum, P. lilacinus* and *V. chlamydosporium* and *B. bassiana* were equally effective in retarding the multiplication of *X. camp.* pv. *citri* compared to control.

Introduction

Citrus cultivation in Pakistan is concentrated in the Punjab province (districts of Sargodha, Faisalabad, Sheikhupura, Multan, Sahiwal and Khaushab) where it is grown on an area of 186.8 thousand hectares with annual production of 1859.2 thousand tons (Anon., 2000). Although citrus is held in great esteem yet its present status is threatened by a number of problems including low production induced by disease. Canker incited by *Xanthomonas campestris* pv. *citri* (Hasse) Dye is one of the major diseases of citrus characterized by small yellowish spots produced on the lower leaves than on upper leaves turning brown and becoming hard and raised from the tissue in later stages of disease development.

Use of resistant rootstock is a valid option, but due to lack of durable resistance in the local/exotic citrus varieties, chemical and biological control is the best alternative to manage citrus canker. Usefulness of some plant diffusates like *Acacia nilotica* (Babool) *Acacia modesta* (Phulai), *Dalbergia sisso* (Shisham) etc. against *X. camp.* pv. *citri* has been determined *in vitro* (Akhtar *et al.*, 1997a, b). The objective of the present studies was to determine the effect of 10 plant extracts and six antagonistic fungi against the multiplication of *X. camp.* pv. *citri* on nutrient agar.

Materials and Methods

Sensitivity of *Xanthomonas campestris* pv. *citri* was studied using inhibition zone technique against ten plant extracts *viz.* Garlic (*Allium sativum*), Babool (*Acacia nilotica*),

Dhatura (*Datura alba*), Shisham (*Dalbergia sisso*), Phulai (*Acacia modesta*), Emblic (*Phyllanthus emblica*), Sufeda (*Eucalyptus camcldulensis*), Bougainvillea (*Bougainvillea spectabilis*), Olive (*Olea europea*) and Pipal (*Ficus religiosa*) and six antagonistic microbes *viz*., *Aspergillus flavus*, *Aspergillus niger*, *Bacaveria bassiana*, *Paecilomyces lilacinus* *Trichoderma* spp. and *Verticillium chlamydosporium*. The leaves of the above mentioned plants were collected from the main campus of University of Agriculture, Faisalabad and cultures of the antagonistic fungi were obtained from culture collection section of the Department of Plant Pathology, U.A., Faisalabad.

For the preparation of aqueous extracts, 75 g fresh leaves of each plant were macerated in 25 mL sterilized water with the help of sterilized pestle and mortar. The macerated leaf extract was first passed through a four layered sterilized muslin cloth and filtered through Whatman's filter paper No. 41. The extract obtained was considered standard (S) (Ilyas *et al*., 1997) and was stored in deep freezer for cold sterilization for further studies in laboratory. Bacterial suspension containing concentration of approximately 10^7 cfu/mL of *Xanthomonas campestris* pv. *citri* was prepared by dilution plate technique (Clifton, 1958) from 48 hours old culture. This suspension was mixed with the lukewarm nutrient agar @ 1 mL/25 mL of medium poured into the sterilized Petri dishes of 9 cm diameter. These Petri dishes were gently shaken to mix the bacteria uniformly in the nutrient agar and then allowed to solidify.

Wells (1 cm diam.) were made in the center of the Petri dishes with the help of sterilized cork borer, and plant extracts were poured into these wells with the help of sterilized syringes. Similarly, 1 cm disc of actively growing culture of each of the fungal antagonist was placed on the nutrient agar in the center of Petri dish. All these Petri dishes were placed in a refrigerator at 4 °C for 24 hours and then transferred to an incubator at 28±2 °C for 48 hours and inhibition zones if any were recorded at intervals of 24, 36 and 48 hours. The experiment was conducted in Completely Randomized Design (CRD) with three replications in each treatment and each replication consisted of three Petri dishes. Control was similarly carried out, except, sterilized water poured in the wells. The data recorded on the inhibition zones were subjected to analysis of variance and the treatment means were compared by LSD test (Steel & Torrie, 1980).

Results and Discussion

Except *Ficus religosa* (Pipal), all the plant extracts at standard concentration reduced the multiplication of *Xanthomonas campestris* pv. *citri* significantly ($p<0.05$) compared to control (Table 1). *Allium sativum* (Garlic) at standard dose exhibited statistically significant inhibition zones compared to other plant extracts. *Acacia nilotica* (Babool) inhibited the growth of *X. camp.* pv. *citri* after garlic extract, while *Datura alba* (Dathura) and *Dalbergia sisso* (Shisham) produced similar effect (Table 1). Inhibition zones exhibited by *Acacia modesta* and *Phyllanthus emblica* (emblic) were found to be statistically similar while those of *Eucalyptus camcldulensis* (Sufeda), *Bougainvillea spectabilis* and *Olea europaea* produced statistically similar effect.

Allium sativum, *Acacia nilotica*, *Datura alba*, *Dalbergia sisso* reduced the growth of *X. camp.* pv. *citri* by 43.0, 25.1, 24.0 and 23.67 % over control. Effectiveness of *Allium sativum* (centrifuged extract) has been proved by Moses & Chandramohan (1993). Akhtar *et al*. (1997a, b) observed the effectiveness of *Acacia nilotica* and *Acacia modesta* against *X. camp* pv. *citri*. *Datura alba* has also been reported to be effective against *X. cam.* pv. *malvacearum* (Ditta *et al*., 1999).

Table 1. *In vitro* evaluation of plant extracts at standard concentrations against *Xanthomonas campestris* pv. *citri*.

Sr. No.	Plant extracts	Inhibitions zones after 48 hours (cm)	Percent decrease over control
1.	*Allium sativum*	3.87 a	43.00
2.	*Acacia nilotica*	2.26 b	24.10
3.	*Datura alba*	2.16 bc	24.00
4.	*Dalbergia sisso*	2.13 c	23.67
5.	*Acacia modesta*	1.60 c	17.78
6.	*Phyllanthus emblica*	0.66 d	07.33
7.	*Eucalyptus cameldulensis*	0.60 dc	06.67
8.	*Bougainvillea spectabilis*	0.53 de	05.89
9.	*Olea europea*	0.10 de	01.11
10.	*Ficus religiosa* (control)	0.00 e	00.00
11.	Untreated control	0.00 e	-----
	LSD	0.6697	-----

Mean values sharing similar letters in a column do not differ significantly at 5 % level of significance.

Table 2. Evaluation of antagonistic fungi against *Xanthomonas campestris* pv. *citri* on nutrient agar.

Sr. No.	Antagonistic fungi	Inhibition zones (cm)	Percent decrease over control
1.	*Aspergillus flavus*	1.73 a	19.22
2.	*Aspergillus niger*	1.27 b	14.11
3.	*Trichoderma* sp.	1.17 b	13.00
4.	*Paecilomyces lilacinus*	1.16 b	12.88
5.	*Verticillium chlamydosporium*	0.77 c	8.55
6.	*Baeuveria bassiana*	0.63 c	7.00
7.	Control	0.00 d	-----
	LSD	0.35	-----

Mean values sharing similar letters in a column do not differ significantly at 5 % level of significance.

Phyllanthus emblica did not inhibit the growth of *X. camp.* pv. *citri* so effectively as described by Akhtar *et al.* (1997b). It retarded the growth of *X. cam.* pv. *citri* only by 7.33 % as compared to control. *Eucalyptus* spp. and *Bougainvillea spectabilis* checked the activity of *X. camp.* pv. *citri* by 6.67 % and 5.89 % over control *in vitro*. These had moderate effect as described by Moses & Chandramohan (1993). *Ficus religiosa* and *Olea europaea* had no effect on the activity of the *Xanthomonas campestris* pv. *citri*.

Among the antagonistic fungi, *Aspergillus flavus* produced maximum inhibition zones than other antagonists as compared to control (Table 2). *Aspergillus niger*, *Trichoderma* spp. and *Paecilomyces lilacinus* exhibited statistically similar effect while inhibition zones produced by *Verticillium chlamydosporium* and *Baeuveria bassiana* were found statistically similar. Thus *Aspergillus niger*, *Trichoderma* spp., *Paecilomyces lilacinus*, *Verticillium chlamydopsorium* and *Beauveria bassiana* retarded the growth of *X. camp.* pv. *citri* 14.11, 13.00, 12.88, 8.55 and 7.00 % over control. All these

antagonistic fungi were evaluated against the bacterium at 30 °C. Masroor & Chaudra (1995) reported that *Aspergillus* spp., produced maximum quantity of antibiotics against *X. camp.* pv. *citri* at 30 °C. *Paecilomyces lilacinus*, a parasite of root knot nematode (Jatala, 1985) produces toxins and antibiotics called as paecilotoxins (Minato *et al.*, 1973) like leucinostatin and lilacin (Arai *et al.*, 1973). *Trichoderma* spp., has been successively used against many plant pathogens *e.g. Armillaria mellea* by combined soil fumigation (Baker & Cook, 1974). *Verticillium chlamydosporium* a parasite of root knot and cyst nematode produces toxins and antibiotics like verticillin A, B, and C, vermicillin and vermiculin (Fuska *et al.*, 1979).

References

Arai, T., Y. Mikami, K. Fukushima, T. Utsumi and Kazawa, 1973. A new antibiotic leucostatin derived from *Penicillium lilacinum*. *J. Antibiotic*. 26: 157-161.

Akhtar, M.A., R. Bhatti and M. Aslam. 1997a. Antimicrobial activity of plant decoction against *Xanthomonas campestris* pv. *citri* on detached leaves. *Trop. Agric.*, 74: 22-28.

Akhtar, M.A., R. Bhatti and M. Aslam. 1997b. Antibacterial activity of plant diffusates against *Xanthomonas campestris* pv. *citri*. *Int. J. Pest Manag.*, 43: 149-153.

Anonymous. 2000. *Agricultural Statistics of Pakistan*. Govt. of Pakistan, Ministry of Food and Agri. Islamabad, 89 pp.

Baker, K.F. and J.R. Cook. 1974. *Biological Control of Plant Pathogens*. S. Chand and Co. Ltd., New Delhi, p. 86-87.

Clifton, C.E. 1958. *Introduction to Bacteria*. 2nd Ed. McGraw Hill Book Co., N.Y., p. 236-279.

Ditta, A., M.A. Khan and M.S. Alam. 1999. *In vitro* sensitivity of *Xanthomonas campestris* pv. *malvacearum* to antagonistic microorganisms and plant extracts. *Proc. 2nd Nat. Conf. Plant Pathology*. Univ. Agri., Faisalabad, p. 120-124.

Fuska, J., A. Fuskova and P. Nemec. 1979. Vermistatin and antibiotic with cytotoxic effects produced from *Penicillium vermiculatum*. *Biologia*, 34: 735-740.

Ilyas, M.B., K. Iftikhar and M. Anwar-ul-Haq. 1997. Effect of different neem products on the vegetative growth and sclerotial production of *Macrophomina phascolina*. *Pak. J. Phytopathol.*, 9: 77-79.

Jatala, P. 1985. Biological control of nematodes. in: *An Advanced Treatise on Meloidogyne*. Vol. I: *Biology and Control*. (Eds.): J.N. Taylor and C.C. Carter. North Carolina State Univ. Raleigh, USA.

Minato, H., M. Matsumoto and T.K. Tyana. 1973. Studies on the metabolites of *Verticillium* spp., verticillin A, B and C. *Chem. Comm.*, p. 1819-1825.

Moses, C.S. and B. Chandramohan. 1993. Potential of neem and other plant extracts for the management of crop diseases. in: *Neem for the Management of Crop Diseases* (Ed.): V. Mariappan. Assoc. Pub. Co., New Delhi, p. 91-97.

Masroor, M.K. and S. Chaudra. 1995. Effect of temperature on antibiotic production by *Aspergillus* spp. antagonistic to citrus canker pathogen. *Biovet*., 6: 65-68.

Steel, R.G.D. and J.H. Torrie. 1980. *Principles and Procedures of Statistics. A Biometrical Approach*. 2nd Ed. McGraw Hill Book Co., N.Y., 633 pp.