



Correlation of environmental variables on canker disease development in commercial citrus cultivars of Pakistan

Muhammad Imran^{1*}, Muhammad Mustafa², Muhammad Azeem³, Muhammad Awais⁴,
M. Aslam khan²

¹Directorate of Agriculture, Pest Warning & Quality Control of Pesticides, Punjab, Lahore, Pakistan

²Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan

³Department of Soil Science, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan

⁴Department of Plant Breeding & Genetic, University of Agriculture, Faisalabad, Pakistan

Key words: Citrus, Citrus canker, Environmental factors, Pakistan, Screening,

<http://dx.doi.org/10.12692/ijb/7.1.1-13>

Article published on July 14, 2015

Abstract

Citrus canker is an extremely costly disease causing worldwide loss of millions of dollars. The onset and development of disease depends upon the favorable environmental conditions. In the present study the crucial environmental variables were correlated. The method provides the ability to examine the evolution of an epidemic in both space and time simultaneously and led to the symptom development. So, study was conducted in research area of Department of Plant Pathology 2008-09. Fifteen commercial citrus varieties were screened against canker disease to find out degree of resistance. Jaffa, pine apple, kinnow, mungal singh, tangerine, succari were found moderately resistant. Five varieties such as chinese lime, musambi, grapefruit, blood red and mayer lime were highly susceptible to canker disease. The susceptible varieties were feutral's early, sweet lime, malta and valentia late showed moderately susceptible response against the canker disease. The disease responses of these citrus varieties were correlated with environmental factors (Air temperature (maximum and minimum), relative humidity (%) and wind velocity (km/hr). Air temperature (maximum and minimum), relative humidity and wind velocity had significant correlations with citrus canker disease development. Rainfall also had significant correlations with citrus canker disease development. Disease started increasing in July and reached maximum incidence in August to October. The current understanding of pathogen and correlation of epidemiological factors help in developing comprehensive management practices to reduce fruit losses.

* Corresponding Author: Muhammad Imran ✉ aazad_1369@yahoo.com

Introduction

Citrus canker is one of the most threatening citrus diseases, affecting all types of important citrus species and varieties in relation to their prevailing climatic conditions. The disease is endemic in India, Japan and other South-East Asian countries except Europe (Das, 2003). In Pakistan Citrus has an important value as fruit plant. The present day citrus is delectable, juicy and seedless is of great nutritional significance as well (Khan *et al.*, 1992). It is used as best source of Vitamin C, sugars, amino acids and other nutrients (Ahmed and Khan, 1999). Pakistan's economy is based on agriculture and fruit production is always an important part of agriculture. The production of all fruits grew by 3.1 percent, out of which production of citrus fruits grew marginally by 0.1 percent (Anonymous, 2009). Citrus is one of the most important productive and highly profitable fruit crop but unfortunately its present status is threatened by a number of problems including low production induced by pests. Of all the agricultural pests and diseases that threaten citrus crop, citrus canker is one of major diseases which adversely affect plant health and fruit development. Disease incidence increases in the presence of citrus leaf miner (CLM), *Phyllocnistis citrella*. The disease caused by the bacterium *Xanthomonas axonopodis* pv. *citri* (Xac)(syn. *X. citri* pv. *citri* Gabriel *et al.*, 1989). The symptoms occurred in the form of necrotic lesions on the leaves, stems and fruits. Severe infections induced defoliation, fruit drop and death of twigs (Schoulties *et al.*, 1987). Bacteria also survive for longer periods of time in lesions on woody branches (Goto, 1992). Bacteria that ooze on the plant surfaces die within hours from desiccation and exposure to direct sunlight (Graham *et al.*, 2000). Exposed bacteria survive only a few days in soil, and a few months in plant refuse that had been incorporated into soil (Graham *et al.*, 1989).

Environmental conditions play a crucial role in the epidemic development of citrus canker disease. Environment variables like temperature and wind speed influenced the citrus canker disease development significantly by the end of September. (Khan *et al.*, 2002; Derso and Sijam, 2007).

Temperature was the greatest factor influencing disease development. At optimum temperatures (25–35°C) there was 100% disease incidence. Maximum disease development was observed at 30–35°C, with up to a 1 2-fold increase in lesion density, a 10-fold increase in lesion size and a 60-fold increase in disease severity.(Christiano *et al.*,2009).

In the recent past, due to changing in temperature and rainfall pattern globally, Pakistan experienced a great variability in temperature resulting into prolonged droughts and uneven rainfall which have adversely affected the disease pattern and their epidemic. The basic theme of the study to characterization of these variation in environmental factors conducive for citrus canker disease development which provide a basis to forecast the disease and issue advance warning to citrus growers for its timely management.

The determination of epidemiological factors i.e. maximum and minimum air temperature, relative humidity, rainfall and wind velocity recorded on daily/weekly basis to find out most favorable conditions for citrus canker development which will be helpful to control the disease by using economical ecofriendly adaptation measures timely.

A better understanding of temperature and precipitation extremes is needed to strategize the resilient resource management for climate change effect mitigation.

Materials and methods

Establishment of disease screening nursery

To evaluate citrus varieties/lines for the relative resistance to canker disease a screening nursery was established in the Plant Pathology Research Area of University of Agriculture, Faisalabad. Varieties encountered for screening were Kinnow(v1), Pine apple(v2), Valencia late(v3), Grape fruit(v4), Blood red(v5), Chinese lime(v6), Mayer lime(v7), Sweet lime(v8), Fuetrell's early(v9), Jaffa (v10), Succari (v11), Tangrin(v12), Mungal Singh (v13), Musambi (v14) and Malta(v15). These varieties were obtained

from the fruit plant nursery of Horticulture Department University of Agriculture, Faisalabad. The experiment was laid out under Randomized Complete Block Design (RCBD). Each variety consisted of twelve plants, which were planted in two rows having six plants in each row. All the recommended agronomic practices were followed to maintain citrus nursery in good condition.

Varietal screening against citrus canker disease

Disease incidence was calculated by using the following formula:

$$\text{Disease incidence} = \frac{\text{No. of infected leaves}}{\text{Total no. of leaves}} \times 100$$

Data regarding disease severity was recorded on weekly basis from December 2008 to December 2009. Plants of each variety were randomly selected and disease response was assessed according to Croxall *et al.* (1952) disease rating scale (Table 1). Difference in disease incidence among the fifteen varieties was determined by LSD at 5% probability level (Steel *et al.*, 1997).

Isolation of bacterium

Leaves exhibiting typical symptoms of citrus canker disease were collected in polyethylene bags and brought to the phytobacteriology laboratory to isolate the bacterium by using the dilution plate technique (Kiralay *et al.*, 1974). First of all, pestles and mortars, petri dishes, medium (Nutrient Agar) and pipettes were autoclaved at 15 lbs pressure (121°C) for 15-20 minutes. Infected leaf tissues were removed using sterilized 10mm cork borer. Leaf discs were surface sterilized in 0.1% mercuric chloride and washed three times in sterilized water. The discs were ground in a sterilized pestles and mortars and the total volume of the mixture adjusted to 10 ml by the addition of sterilized water, followed by the preparation of tenfold dilutions from the mixture. 1 ml of each dilution was poured into a petri dish and Luke warm (45°C) nutrient agar was poured on to it. Each petri dish was shaken. Petri dishes were incubated at 30°C±2°C. Yellow and round colonies appearing after

36 hours incubation were transferred to agar slants to prepare pure cultures. The bacterium was identified using morphological and biochemical characteristics (Breed *et al.*, 1989). Stock cultures of the bacterium were maintained on nutrient agar in culture tubes at 4°C.

Pathogenicity Test

The isolated bacterium was examined for pathogenicity on healthy plants. Two years old ten citrus plants of variety Mayer lemon and Grapefruit were obtained from nursery of Department of Horticulture University of Agriculture, Faisalabad. These plants were transplanted into pots containing field soil disinfected with 5% formalin. The bacterium from stock culture was multiplied on nutrient agar by incubating for 48 hours at 30°C±2°C. An aqueous suspension of the bacterium having a concentration of approximately 10⁸ cells/ml was prepared by plate count method (Kiraley *et al.*, 1974).

Just before inoculation, plants were irrigated and covered with polythene bags for two hours to create conditions of high humidity and placed under sunlight to allow the stomata to open to the maximum (Weindling, 1948; Gunn, 1962). The abaxial surface of the leaves was inoculated using a spraying machine at a pressure of 1.1kg/cm² until the tissue showed water soaking. In control the plants were sprayed only with sterilized water. The plants were kept under observation for two weeks in the greenhouse and symptoms, if any were recorded. Reisolation the bacterium from diseased tissue was carried out in the way as described above and morphological characteristics (Breed *et al.*, 1989) of the isolates compared with the original culture of bacterium used in inoculations. The bacteria showing similar colony characters that of the original culture were considered to be pathogenic.

Epidemiological studies

The data of different environmental factors (maximum, minimum temperature, relative humidity, wind velocity and rainfall) during the disease rating period were obtained from the

Department of Crop physiology, University of Agriculture, Faisalabad.

Statistical analysis

Data regarding above mentioned environmental factors were correlated with the disease intensity. The relationship of environmental factors with citrus canker disease intensity was determined through regression analysis (Steel *et al.*, 1997).

Results

Reaction of different citrus cultivars/varieties

Table 1. Disease rating scale used to determine the level of resistance or susceptibility to citrus canker.

Grade	Disease Severity (%)	Response
0	00-00	Highly Resistant
1	01-05	Resistant
3	06-10	Moderately Resistant
5	11-15	Moderately Susceptible
7	16-25	Susceptible
9	26 and above	Highly Susceptible

C. sinensis cv. valentia late showed moderately susceptible response against the disease with disease rating of 5 while *C. sinensis* cv. jaffa, *C. sinensis* cv. pine apple, *C. reticulata* cv. tangerine, *C. reticulata*

against canker disease

Fifteen varieties/lines of citrus were evaluated for relative resistance to canker disease in natural environment. All the varieties showed different levels of disease development. *C. limonia* cv. china lemon, *C. sinensis* cv. Succari, *C. paradise* cv. grapefruit, *C. sinensis* cv. blood red and *C. limonia* cv. mayer limone were highly susceptible with disease ratings of 9, while *C. reticulata* cv. feutral's early, *C. reticulata* cv. malta and *C. limettioides* cv. sweet lemon were susceptible with disease ratings of 7.

cv. kinnow, *C. sinensis* cv. succari, *C. reticulata* cv. mungal singh were moderately resistant with disease ratings of 3 (Table 2).

Table 2. Level of resistance/susceptibility to canker disease exhibited by various citrus varieties.

Sr.#	Varieties/cultivars	Disease incidence (Mean)	Ratings	Response
1	Jaffa	8.01 k*	3	MR
2	Pine apple	8.29 k	3	MR
3	Kinnow	8.60 jk	3	MR
4	Mungal singh	8.98 ij	3	MR
5	Tangerine	9.52 hi	3	MR
6	Succari	9.78 h	3	MR
7	Valentia late	10.90 g	5	MS
8	Feutral'early	18.06 f	7	S
9	Sweet lime	20.86 e	7	S
10	Malta	20.97 e	7	S
11	Chinese lime	25.73 d	9	HS
12	Musambi	27.83 c	9	HS
13	Grapefruit	30.15 b	9	HS
14	Blood red	30.37 b	9	HS
15	Mayer lime	31.50 a	9	HS

*Means sharing similar letters do not differ significantly ($P > 0.05$).

MR = Moderately resistant

MS = Moderately susceptible

S = Susceptible

HS = Highly susceptible.

Table 3. Correlation among different environmental variables and disease index (all data).

Environmental Parameters	Disease Index
Maximum Temperature	0.890**
	0.003
Minimum Temperature	0.662**
	0.001
Relative Humidity	-0.529*
	0.033
Rainfall	0.551*
	0.041
Wind Speed	0.502*
	0.013

Overall correlation of environmental factors with disease index

The overall correlation of citrus canker disease index with maximum and minimum temperature was positive which indicate that with increase in maximum and minimum temperature the disease was increased significantly (Table 3). Citrus canker disease development in ten varieties out of fifteen varieties showed the significant correlation with maximum temperature and five varieties exhibited the non significant correlation (Table 4) similarly

eleven varieties showed the significant correlation with the minimum temperature and four varieties showed non significant correlation (Table 4). There was significant correlation between citrus canker and relative humidity. It was negatively correlated with the relative humidity which indicates that with increase in the relative humidity disease was decreased significantly (Table 3). Eight varieties showed the significant correlation with relative humidity and seven varieties showed the non significant correlation (Table 4).

Table 4. Correlation among different environmental factors and disease index for different varieties (all data).

Sr.no.	Variety / line	Temperature (°C)		R.H.	Rainfall	Wind speed
		Max.	Min.			
1	Jaffa	0.708*	0.619*	-0.605*	0.763**	0.157
		0.013	0.021	0.015	0.000	0.271
2	Kinnow	0.608**	0.540*	0.116	0.110	0.723**
		0.003	0.038	0.417	0.440	0.000
3	Mungal singh	0.530*	0.749**	-0.552**	0.745**	0.151
		0.018	0.007	0.001	0.004	0.290
4	Pine apple	0.735**	0.701*	-0.501**	0.729	0.513*
		0.000	0.034	0.004	0.003	0.023
5	Tangerine	0.663*	0.640**	0.616*	0.140	0.619*
		0.035	0.009	0.019	0.326	0.051
6	Succari	0.521	0.540*	0.090	0.653**	0.112
		0.242	0.010	0.532	0.35	0.436
7	Valentia late	0.634	0.231	-0.265	0.749**	0.533**
		0.098	0.102	0.060	0.000	0.000
8	Feutral'early	0.501**	0.554*	-0.337*	0.108	0.613*
		0.000	0.031	0.016	0.450	0.014
9	Malta	0.493**	0.624*	-0.428*	0.530*	0.165
		0.000	0.014	0.002	0.037	0.249
Citrus						
10	Limettioides	0.703	0.209	-0.091	0.509**	0.605*
		0.154	0.141	0.526	0.000	0.014
11	China lemon	0.566*	0.516*	-0.281*	0.668*	0.512*
		0.016	0.021	0.046	0.035	0.014
12	Musambi	0.496	0.603*	-0.110	0.752**	0.143
		0.169	0.040	0.442	0.004	0.315
13	Citrus paradise	0.443**	0.123	-0.634*	0.042	0.612**
		0.001	0.638	0.013	0.768	0.002
14	Blood red	0.670**	0.039	0.030	0.549**	0.524**
		0.002	0.788	0.837	0.007	0.001
15	Mayer lemon	0.246	0.517*	-0.022	0.764**	0.027
		0.081	0.042	0.880	0.003	0.850

Upper values indicated Pearson's correlation coefficient. Lower values indicated level of significance at 5% probability. * = Significant (P<0.05); ** = Highly significant (P<0.01)

Rain fall showed the significant correlation with the disease development (Table 3) Citrus canker was significantly increased with increase in rainfall and out of fifteen varieties ten varieties showed the significant correlation with rainfall (Table 4). There

was significant correlation between citrus canker and wind speed (Table 3) Citrus canker was significantly increased with increase in wind speed and out of fifteen varieties nine varieties showed the significant correlation with the wind speed (Table 4).

Table 5. Correlation among different environmental variables and disease index of months (January to April).

Environmental Parameters	Disease Index
Maximum Temperature	0.622
	0.087
Minimum Temperature	0.578
	0.059
Relative Humidity	-0.193
	0.628
Rainfall	0.110
	0.321
Wind Speed	0.098
	0.429

Correlation of environmental variables of January to April with citrus canker disease

It was observed in the overall correlation that environmental variables showed non-significant correlations with the disease development as shown in (Table 5).

The varietal based correlation showed that only four varieties in first four month of data recording during

the experiment showed the significant correlation with the maximum temperature and most of varieties showed the non significant correlation. Only three varieties showed the significant correlation with the minimum temperature and three varieties showed the significant correlation with the wind speed. All varieties the varieties were non-significantly correlated with the relative humidity and rainfall shown in Table (6).

Table 6. Correlation among different environmental factors and disease index for different varieties (January to April).

Sr.no.	Variety / line	Temperature (°C)		R.H.	Rainfall	Wind speed
		Max.	Min.			
1	Kinnow	0.557	0.532	-0.171	0.088	0.073
		0.270	0.356	0.231	0.541	0.610
2	Jaffa	0.722*	0.341	-0.167	0.089	0.244
		0.011	0.088	0.242	0.535	0.084
3	Pine apple	0.639	0.639*	-0.154	0.215	0.154
		0.091	0.047	0.280	0.129	0.281
4	Succari	0.524	0.614	-0.139	0.065	0.706*
		0.113	0.131	0.332	0.649	0.015
5	Mungal singh	0.616	0.037	-0.228	0.029	0.011
		0.416	0.799	0.107	0.842	0.941
6	Tangerine	0.526	0.520*	-0.069	-0.016	0.220
		0.111	0.047	0.631	0.911	0.121
7	Malta	0.710*	0.629	0.001	0.048	0.141
		0.028	0.106	0.996	0.737	0.322
8	Valentia late	0.177	0.408	0.028	0.094	0.118
		0.215	0.143	0.845	0.510	0.411
9	Feutral's early	0.526*	0.524	-0.264	0.152	0.549*
		0.012	0.968	0.061	0.288	0.018
10	China lemon	0.234	0.466	-0.110	0.021	0.067
		0.098	0.244	0.444	0.886	0.640
11	Citrus paradise	0.345	0.568	-0.031	0.139	0.176
		0.311	0.238	0.830	0.331	0.216

12	Musambi	0.483	0.680	-0.208	0.136	0.178
		0.199	0.206	0.144	0.340	0.211
	Citrus					
13	Limettioides	0.563	0.808*	0.393	0.069	0.505*
		0.456	0.031	0.267	0.633	0.033
14	Blood red	0.652*	0.233	-0.254	0.261	0.425
		0.019	0.589	0.072	0.064	0.369
15	Mayer lemon	0.253	0.235	-0.243	0.120	0.369
		0.886	0.125	0.086	0.402	0.587

Upper values indicated Pearson's correlation coefficient. Lower values indicated level of significance at 5% probability. * = Significant ($P < 0.05$); ** = Highly significant ($P < 0.01$).

Correlation of environmental variables of May to August with citrus canker disease

It was observed in the overall correlation that environmental variables showed significant

correlations with the disease development. There was significant change in the disease index in the months of data recording during the May to August shown in the table (Table 7).

Table 7. Correlation among different environmental variables and disease index of months (May to August).

Environmental Parameters	Disease Index
Maximum Temperature	0.892**
	0.007
Minimum Temperature	0.759*
	0.018
Relative Humidity	-0.516**
	0.002
Rainfall	0.528*
	0.029
Wind Speed	0.631*
	0.037

It was found that there was highly significant correlation of disease with maximum temperature and relative humidity (Table 7). Other environmental variables also significant correlated with the disease development. All the environmental variables showed the positive correlation with the disease development

except the relative humidity which was negatively correlated with the disease development. Disease increased with increase in maximum, minimum temperature, wind speed and rainfall while disease decreased with increase in relative humidity (Table 8).

Table 8. Correlation among different environmental factors and disease index for different varieties (May to August).

Sr.no.	Variety / line	Temperature (°C)		R.H.	Rainfall	Wind speed
		Max.	Min.			
1	Kinnow	0.689*	0.536	-0.776**	0.668	0.673
		0.021	0.356	0.001	0.541	0.610
2	Jaffa	0.758	0.561*	-0.167	0.639*	0.244
		0.099	0.021	0.242	0.023	0.084
3	Pine apple	0.789*	0.259	-0.569*	0.715	0.694**
		0.035	0.072	0.021	0.139	0.016
4	Succari	0.594	0.766*	-0.139	0.568*	0.304
		0.123	0.036	0.332	0.039	0.466
5	Mungal singh	0.693*	0.337	-0.546*	0.269	0.568*

		0.043	0.799	0.028	0.832	0.013
6	Tangerine	0.527	0.280*	-0.869*	-0.236	0.220
		0.131	0.047	0.046	0.911	0.121
7	Malta	0.823**	0.846**	0.659**	0.645**	0.741**
		0.000	0.000	0.000	0.000	0.000
8	Valentia late	0.197	0.526*	0.028	0.194	0.118
		0.265	0.011	0.845	0.210	0.411
9	Feutral's early	0.859**	0.876**	-0.726*	0.776*	0.732*
		0.000	0.000	0.025	0.022	0.019
10	China lemon	0.694	0.176	-0.116	0.621	0.467
		0.098	0.344	0.444	0.886	0.640
11	Citrus paradise	0.743*	0.756**	-0.599**	0.789*	0.709*
		0.018	0.000	0.001	0.033	0.023
12	Musambi	0.523	0.696*	-0.602	0.725*	0.378
		0.199	0.012	0.144	0.023	0.211
	Citrus					
13	limettioides	0.646	0.553**	-0.373**	0.169	0.505**
		0.060	0.000	0.007	0.333	0.000
14	Blood red	0.793**	0.721**	-0.676**	0.543*	0.689**
		0.000	0.000	0.001	0.041	0.002
15	Mayer lemon	0.243	0.637**	-0.689	0.710*	0.612**
		0.529	0.000	0.086	0.031	0.000

Upper values indicated Pearson's correlation coefficient. Lower values indicated level of significance at 5% probability. * = Significant ($P < 0.05$); ** = Highly significant ($P < 0.01$).

Correlation of environmental variables of September to December with citrus Canker disease

It was found that among the environmental variables the maximum temperature and rainfall showed the

significant correlation with the disease development (Table 9).

Table 9. Correlation among different environmental variables and disease index of months (September to December).

Environmental Parameters	Disease Index
Maximum Temperature	0.672*
	0.047
Minimum Temperature	0.692
	0.073
Relative Humidity	-0.319
	0.439
Rainfall	0.459*
	0.037
Wind Speed	0.077
	0.458

The varietal based correlation showed that five varieties in months of September to December data recording during the experiment showed the significant correlation with the maximum temperature and only three varieties showed the

significant correlation with the minimum temperature. And four varieties showed the significant correlation with the relative humidity and rainfall. Only two varieties showed the significant correlation with the wind speed (Table 10).

Table 10. Correlation among different environmental factors and disease index for different varieties (September to December).

Sr.no.	Variety / line	Temperature (°C)		R.H.	Rainfall	Wind speed
		Max.	Min.			
1	Kinnow	0.757*	0.532	-0.156	0.068	0.062
		0.046	0.356	0.231	0.541	0.621
2	Jaffa	0.548	0.341	-0.162	0.568*	0.268
		0.079	0.088	0.242	0.025	0.084
3	Pine apple	0.639	0.654	-0.543*	0.215	0.256
		0.091	0.072	0.021	0.129	0.283
4	Succari	0.724	0.714	-0.168	0.065	0.184
		0.113	0.131	0.332	0.649	0.436
5	Mungal singh	0.563*	0.837	-0.358	0.029	0.011
		0.027	0.799	0.107	0.842	0.941
6	Tangerine	0.226	0.580*	-0.621*	0.316	0.430
		0.111	0.047	0.031	0.911	0.168
7	Malta	0.409	0.229	0.001	0.648*	0.198
		0.141	0.106	0.369	0.026	0.339
8	Valentia late	0.457	0.208	0.246	0.026	0.118
		0.215	0.143	0.845	0.510	0.411
9	Feutral's early	0.625*	0.690	-0.764*	0.052	0.289
		0.015	0.548	0.041	0.288	0.239
10	China lemon	0.534	0.569*	-0.110	0.121	0.067
		0.098	0.011	0.454	0.886	0.640
11	Citrus paradise	0.645	0.533*	-0.031	0.569*	0.176
		0.311	0.029	0.880	0.035	0.216
12	Musambi	0.583	0.180	-0.208	0.266	0.436
		0.199	0.206	0.144	0.350	0.229
Citrus						
13	limettioides	0.680*	0.563	-0.375*	0.069	0.572*
		0.038	0.124	0.004	0.633	0.016
14	Blood red	0.758	0.530	-0.238	0.450*	0.297
		0.475	0.026	0.072	0.000	0.321
15	Mayer lemon	0.684*	0.637	-0.243	0.140	0.426*
		0.021	0.256	0.268	0.222	0.000

Upper values indicated Pearson's correlation coefficient. Lower values indicated level of significance at 5% probability. * = Significant ($P < 0.05$); ** = Highly significant ($P < 0.01$).

Discussion

Citrus canker disease, caused by *X. axonopodis pv. citri* has re-emerged as potential threat to citrus plantation throughout the world including Pakistan (Civerolo, 1984) 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). Genetic resistance probably is the only durable and long lasting solution to citrus canker diseases. The short-term solution should be screening of available

germplasm for relative susceptibility, as in this study and to identify low rating variations for breeding manipulation. Out of 15 cultivars *C. sinensis* cv. jaffa, *C. sinensis* cv. pine apple, *C. reticulata* cv. tangerine, *C. reticulata* cv. kinnow, *C. sinensis* cv. succari, *C. reticulata* cv. mungal singh exhibited resistant response, whereas *C. limonia* cv. china lemon, *C. sinensis* cv. succari, *C. paradise* cv. grapefruit, *C. sinensis* cv. blood red and *C. limonia* cv. mayer lemone showed high susceptibility to citrus canker disease. (Table 2).

Evaluation of different varieties of citrus to find

resistant source against canker has been reported by many research workers and the results of present study were agreed with Wang and Chung, 1991) who observed that Xac occurred widely on grapefruit, sweet orange, lemon and other citrus species. Ayub *et al.*, 1996) concluded that isolates of Xac when inoculated into various citrus hosts, *C. aurantifolia*, *C. aurantium*, *C. paradisi*, *C. limon*, *Poncirus trifoliata* and *C. sinensis*, these hosts showed susceptibility in decreasing order. *C. reticulata* was found to be resistant. Civerolo, 1984) reported that among commercial citrus varieties and rootstocks, Asiatic citrus canker (ACC) was most severe on *C. paradise*, *C. aurantifolia*, *C. limettioides*, *Poncirus trifoliata* and their hybrids because of high susceptibility. Leite and Mohan, 1990) reported that there was a wide range of variability for resistance to citrus canker disease in the citrus germplasm. The commercial citrus canker - resistant cultivars of sweet orange, mandarins and tahiti lime are usually recommended for planting. According to Pavan *et al.*, 2007), mandarins and tangerines were recognized as tolerant to Asiatic citrus canker disease while Sweet orange exhibited the susceptible response among commercial varieties. The results of screening were also matched with that of Atiq *et al.*, 2007) screened fifteen citrus cultivars for the source of resistance against citrus canker disease incited by (*Xanthomonas campestris* pv. *citri*) and concluded that *Citrus sinensis* cv. jaffa exhibited resistance response while *Citrus paradise*, *Citrus sinensis* cv. blood red, *Citrus limonia* cv. mayer lemon showed highly susceptible expression. *Citrus sinensis* cv. valentia late, *Citrus reticulata* cv. feutral's early showed moderately resistant while *Citrus reticulata* cv. malta, *Citrus limettioides*, *Citrus limonia* cv. china lemon, *Citrus sinensis* cv. musambi were found moderately susceptible toward canker disease. No citrus cultivar was found immune.

The overall correlation of citrus canker disease incidence with the maximum and minimum temperatures was significant. These results are agreed with Verniere, 2003) who studied temperature along with different environmental factors (wind velocity, relative humidity, rainfall, sun shine, clouds) had

great effect on the citrus canker disease development and concluded that air temperature (maximum and minimum) was the most significant factor in disease development described by AUDPC. Koizumi, 1976) also suggested that maximum (36-38°C) and minimum (13°C) temperatures played a significant role in the development of citrus canker. Further evidence was provided by Khan *et al.*, 1992) who stated that a low temperature range (8-11°C) in the month of January and February during the year played a significant role in the spread of citrus canker disease. During the whole year disease incidence showed significant correlations with maximum, minimum temperature and these findings were according to the research work of Zhihua *et al.*, 2001) who reported that citrus canker appeared in late April and early May; the most severe period being mid May to early June. Occurrence was correlated with temperature in mid -April; the higher the temperature, the earlier the occurrence. A significant correlation was also found between wind velocity and citrus canker disease development. These results were according to Palazzo *et al.*, 1987) who reported that citrus canker caused by Xac spread rapidly in summer favored by southeast and northwest winds, at 25°C or higher and with rain. Khan *et al.*, 2002) reported that out of six-environment variables assessment, minimum temperature and wind speed influenced citrus canker disease development most significantly. Wind-driven rain splash played a dynamic role in dispersal of Xac (Gottwald *et al.*, 1997; Rehman and Khan, 2000). Gottwald, *et al.*, 2002) reported that rapid spread of disease across regions was in response to rainstorms with wind.

There was significant correlation between rainfalls with citrus canker incidence. These results agreed with those Gottwald, 1997) who observed that once disease established, the most important way of dispersal were rain splash and wind. Individual meteorological events, such as thunderstorms, tropical storms and hurricanes had contributed to medium to long distance dispersal of bacteria from the original focus. Relative humidity was negatively correlated with citrus canker disease development

and it was agreed with Ikram, 2001) who found that with increasing in relative humidity there was reduction in citrus canker disease development, contrast to Sothisorubini *et al.*, 1986) reported that ideal conditions for infection of citrus plants by Xac were temperature of 30°C and 100% relatively humidity.

Conclusion

Citrus canker continues to be the cause of worldwide concern as a potentially hazardous threat to citriculture. A better understanding of the pathogenic specialization and proper identification of Xac strains are needed. Control of citrus canker in the areas where disease is present, the most effective disease management strategy is the use of disease resistant varieties, characterization of environmental factors conducive for citrus canker disease may provide a basis to forecast the disease and early warning to citrus growers for its timely management are recommended because frequent use of chemicals is neither economical nor environment friendly.

Acknowledgments

We deeply thanks to Prof. Dr. Muhammad Aslam khan, department of plant pathology, UAF for his kind supervision.

References

- Amad R, Khan HH.** 1999. Citrus decline problems in the Punjab: A review Pp 20-22. In: 2nd Nat. Conf. PL. Path. Univ. Faisalabad. Pakistan.
- Anonymous.** 2000. Agricultural Statistics of Pakistan. Govt. of Pakistan. Ministry of Food and Agric. Islamabad 89. p.
- Atiq M, Khan MA, Sahi T.** 2007. Screening off citrus germplasm for the source of resistance against canker disease caused by *Xanthomonas axonopodis* pv *citri*. Pakistan Journal of Phytopathology **19(2)**, 222-226.
- Ayub M, Jahangir HS, Mumtaz K, Amin M.** 1996. Pathogenic variation and host range of *X. campestris* pv. *citri* isolates. Pakistan Journal of Phytopathology **18**.
- Breed RS, Murry EGD, Smith NR.** 1989. Bergey's Manual of Systemic Bacteriology. (Eds.S. T. Williams, M. E. Sharpe, and J. G. Holt). Williams and Wilkinson Co. Baltimore **4**.
- Christiano RSC, Dalla Pria M, Jesus Junior WC, Parra JRP, Amorim L, Bergamin Filho A.** 2007. Effect of the citrus leaf-miner damage, mechanical damage and inoculum concentration on severity of symptoms of Asiatic citrus canker in Tahiti lime. Crop Protection **26**, 59–65.
<http://dx.doi.org/10.1016/j.cropro.2006.03.016>
- Civerolo EL.** 1984. Bacterial canker disease of citrus. Journal Rio Grande Valley Horticulture Society **37**, 127-146.
<http://dx.doi.org/10.1094/PHP-2002-0812-01-RV>.
- Das A K, Singh S.** 2003. Integration of chemicals and cultural practices for management of bacterial canker (*Xanthomonas axonopodis* pv *citri*) in acid lime (*Citrus aurantifolia*). Indian Journal of Agriculture Sciences **73**, 570-571.
nrc Citrus.nic.in/index.php?c=pages&m=index&id=71
- Derso E, Sijam K.** 2007. Citrus canker: a new disease of Mexican lime (*Citrus aurantifolia*) and sour orange (*C. aurantifolia*) in Ethiopia. Fruits **62**, 89-98.
www.fruitsjournal.org/articles/fruits/abs/2007/02/i7202/i7202.html
- Gabriel DW, Kingsley MT, Hunter JE, Gottwald TR.** 1989. Reinstatement of *Xanthomonas citri* (Hasse) and *X. phaseoli* (Smith) to species and reclassification of all *X. campestris* pv. *citri*. Strains. International Journal of Systematic Bacteriology **39**, 14-22.
<http://dx.doi.org/10.1099/00207713-39-1-14>
- Goto M.** 1992. Citrus canker in plant diseases of

international importance Vol. III. Disease of fruit crops. Facul. Agric., Shizouka Univ., 836 Ohya, Shizouka, 422 Japan (Review Plant Pathology **52(10)**, 6997.

Gottwald TR, Graham JH, Schobert TS. 1997. An epidemiological analysis of the spread of citrus canker in Urban Miami, Florida, Deptt. Agri., U.S.A. Fruit Paris **52(6)**, 383-390.

https://www.msu.edu/user/staatz/An_Epidemiological.pdf

Gottwald TRX, Sun Riley T, Graham JH, Ferrandino F, Taylor EL. 2002. Georeferenced spatiotemporal analysis of the urban citrus canker epidemic in Florida. Phytopathology **92(4)**, 361.

<http://dx.doi.org/10.1094/PHYTO.2002.92.4.361>.

Graham JH. 1989. Population dynamics and survival of *Xanthomonas campestris* in soil in citrus nurseries in Maryland and Argentina. Plant Disease **73**, 423-427.

www.apsnet.org/publications/PlantDisease/.../PlantDisease73n05_423.pdf

Graham JH, Gottwald TR, Riley TD, Cubero J, Drouillard DL. 2000. Survival of *Xanthomonas campestris* pv. *citri* (Xcc) on various surfaces and chemical control of Asiatic citrus canker (ACC). Proc. Int. Citrus Canker Res. Workshop, June 20-22, 2000, Ft. pierce, Florida, p.7.

Gunn RE. 1962. Bacterial blight of cotton. A seedling inoculation technique. Emp Cott Gr Rev **39**, 188-190.

Ikram F. 2001. Relationship of environmental conditions with citrus canker disease and its management. Msc. Thesis Deptt. of Plant Pathology, Uni. Agri., Faisalabad. 36-38 P.

Kiralay Z, Klement Z, Ealymasy F, Vaaras J. 1974. Methods in Plant Pathology. Elsevier Scientific Pub. Co., New York.

Khan IA, Jaskani MJ, Ali SNH. 1992. Breeding for seedless Kinnow, a Progress Report. In: Proceed. 1st Inter. Sem. Citriculture in Pakistan. Dec. 2-5. University of Agriculture Faisalabad 103-55 P.

Khan MA, Khan MM, Haq MI, Javed N. 1992. Antibacterial activity of various toxicants against *Xanthomonas campestris* pv. *citri* for the control of citrus canker disease: Proc. 1st Int. Sem. Citriculture in Pakistan, Dec. 2-5, 311-314 P.

Koizumi M, Kimijima E, Tsukamoto T, Togawa M, Masui S. 1996. Dispersion of citrus canker bacteria in droplets and prevention with windbreaks. Proc. Int. Soc. Citric **1**, 340-344.

www.crec.ifas.ufl.edu/societies/ISC/author/southafrica.shtml

Khan MA, Rashid A, Ikram F. 2002. Weekly minimum temperature one variable model to predict citrus canker disease. Pakistan Journal of Phytopathology **14(1)**, 7-10.

Leite RP, Mohan SK. 1990. Integrated management of the citrus bacterial canker disease caused by *Xanthomonas campestris* pv. *citri* in the state of Parana, Brazil. Crop Protection **9**, 3-7.

Pavan A, Calixto MC, Cardoso SC, Mendes BMJ, Filho AB, Lopes JRS, Carvalho CRD, Filho EDAM. 2007. Evaluation of 'Hamlin' sweet orange + 'Montenegrins' mandarin somatic hybrid for tolerance to *Xanthomonas axonopodis* pv. *citri* and *Xylella fastidiosa*. Sci. Horticult.

Palazzo DA, Noguciro EMD, Cirerolo LC, Montovanello CM. 1987. Epidemiological studies on citrus canker (*Xanthomonas campestris* pv. *citri*) progress of disease in Lime. In the state of Biolo Sao Paulo Brazil **1**, 133-140.

Rehman H, Islam KS, Jahan M. 2002. Seasonal incidence and extent of damage caused by citrus leaf miner, (*Phyllocnistis citrella*, Stainton) infesting lemon. Pakistan Journal of Scientific and Industrial

Research **48(6)**, 422-425.

Schoulties CL, Civerolo EL, Miller JW, Stall RE, Krass CJ, Poe SR, Ducharme EP. 1987. Citrus canker in Florida. *Plant Disease* **71**, 388-395.

Sothiosorubini M, Sundersan RV, Sivapalan A. 1986. Studies on *Xanthomonas citri* (Hasse) Dowson causing canker disease of citrus. *Vingnanam Journal of Sciences Serilanka* **1(1)**, 19-25.

http://dx.doi.org/193.43.36.125/XML_Output/2012/OV/OV2012030630003063.xml

Steel RGD, Torrie JH, Dickey DA. 1997. Principles and Procedures of Statistics. A Biometrical Approach. 3rd edit. Mc Graw Hill Book Co., New York.

Verniere CJ, Gottwald'and TR, Pruvost O.

2003. Disease Development and symptom expression of *Xanthomonas axonopodis* pv. *citri* in various citrus plant tissues. *Phytopathology* **93(7)**, 832-43.

<http://dx.doi.org/10.1094/PHTO.2003.93.7.832>

Wang LY, Chung KC. 1991. A Supplement list of *Xanthomonas campestris* pv. *citri* strains isolated in Taiwan. *Plant Protection Bulletin Taiwan* **33(3)**, 301-304.

Weindling R. 1948. Bacterial blight of cotton under conditions of artificial inoculation. U. S. D. A. Washington, D.C. Tech Bull. No **956**, 204.

www.nal.usda.gov/ref/USDApubs/tb.htm

Zhi Hua F, Zhan Hua X, Zhu F, Zhu X. 2001. Study on the occurrence of citrus canker and its control. *South China Fruits* **30**, 4-15.

eurekamag.com/research/003/572/003572270.php