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GPS Based Surveillance and Chemothrerapeutic Management of Citrus Canker Disease and Leaf Miner in Relation to Prevailing Environmental Conditions in Sargodha District

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Abstract

In order to construct a disease management strategy, citrusorchards of different tehsils of district Sargodha *viz.* Bhalwal, Kot Momin, SillanWali, Shahpur, Sahiwal and Sargodha were surveyed to measure incidence of canker and citrus leaf miner population in relation to prevailing environmental conditions by using global positioning system (GPS). GPS data was collected from Globalnaya Navigazionnaya Sputunikovaya Sistema (GLONASS) a program of Russian space agency which offer its services on mobile app. The data was uploaded on google maps to draw an incidence map. Maximum disease incidence was observed in Kot Momin mainly, Chak No 21 S.B (32.16078, 73.02801) and Mela (32.16976, 73.13783). Citrus leaf miner showed maximum prevalence at Bhalwal mainly in Lilyani (32.21312, 72.94722). Except relative humidity, all environmental conditions were found significantly correlatedwith both disease incidence and citrus leaf miner population at all six localities. With every one unit increase in minimum and maximum temperature, rain and wind speed disease incidence and citrus leaf miner population also increased. Among chemicals, Streptomycine + Copper oxychloride @ 1 + 3g/L and match 50 EC were found most effective in controlling citrus canker and citrus leaf miner population as compared to control and all other chemicals.

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Introduction

Citrus orchards are affected by number of insect pests and diseases which are very detrimental for the citrus industry. A numbers of insect species including Citrus psylla (*Diaphorina citri* Kuwayama), Citrus caterpillar (*Papiliodemoleus* L.), lance nematode *Hoplolaimus indicus* Sher and citrus nematode *Tylenchulu semipenetrans* attack on citrus fruits all over the world.Among these, citrus leaf miner (*Phyllocnistiscitrella* Stainton) is the most destructive (Pena *et al.,* 2002; Hall *et al.,* 2013). Moreover, the basic reasons of low yield and rate of export are lack of improved production technology, low quality fruit, lack of actual knowledge of developing and producing good quality fruits, inadequate infra-structure processing facilities, and the foremost thing is improper management of the insect pest, disorders and the diseases (Schueller *et al.,* 1999). Citrus is susceptible to a number of different diseases like Anthracnose, citrus scab, citrus slow decline, die back, citrus wither tip, damping off, leaf spot diseases, citrus greening, CTV, decline disorders and citrus canker. Among all of these citrus canker caused by *Xanthomonas axonopodis* pv. *citri*(*Xac*) has significant economic importance (Graham *et al.,* 2004). Due to disease incidence 257000 citrus plants were discarded in Florida during1915-1933. Distinctively elevated necrotic lesions are formed on fruits, stems and leaves due to itsinfection. *Xac* perseveres from one growing season in the form of lesions and transferred to next growing season (Goto 1992). Bacterium remains viable as long as the host cells in the neighborhood of lesions are alive. Extracellular polysaccharide condenses the bacterium cell exuded on to the scrape surface, aiding dispersion in pathogen (Goto and Hyodo, 1985).

To control citrus canker in areas where the disease does not exist; introduce the quarantine or governing program to forbid the exposure of diseased citrus plant material and fruits to the healthy plants. To resolve the problem in the regions where citrus canker disease is present, different disease management strategies including timely application of protective chemicals, bio-chemicals and plant extracts but the best strategy includes use of resistant varieties, avoid mono-cultures and control CLM (Das 2003).

Citrus life miner (*Phyllocnistis citrella*) is an aggressive pest of citrus plant. It is commonly observed that CLM is an efficient vector of citrus canker disease. The higher incidence of citrus canker was observed with the increase in population of *P. Citrilla* on host citrus plants (Cook, 1988; Chagas *et al.,* 2001). Survey of citrus orchards (Malan*et al.,* 2011) and finding correlation with various environmental factors are helpful for researchers and policy makers for developing different management strategies (Arora *et al.,* 2013). Kalita *et al.*(1995) recorded the maximum incidence of citrus canker in August when the rainfall, temperature and relative humidity were more in this month. Arora *et al.*(2013) correlated the environmental conditions conducive for disease incidence and indicated that rainfall, relative humidity and minimum temperature have positive correlation while maximum temperature have negative correlation with the disease development.

Keeping in view all above mentioned facts the main objectives of current study were (1) to conduct a comprehensive survey of citrus orchards in district Sargodha for the identification of citrus canker and leaf miner incidence and (2) correlate disease incidence with prevailing environmental conditions at that area and (3) to evaluate different chemicals against citrus canker and citrus leaf miner disease management.

Materials and methods

In order to record the disease incidence, survey was conducted in citrus orchards on Kinnow, *Citrus reticulata* varieties at district Sargodha and its five Tehsils including Sahiwal, Bhalwal, Sillan Wali, Shahpur and Kot Momin (Table 1).

Three citrus orchards of each tehsils were randomly selected and status of the disease was recordby using the following formula (Johnston & Booth 1983).

$$Disease\ incidence = \frac{No.\ of\ infected\ plants}{Total\ No.\ of\ Plants} \times 100$$

Pathogen study

The suspected canker infected plant leaves samples were taken from different tehsils of Sargodha. After washing small pieces were made by seizer and were surface sterilized in 0.1% $HgCl_2$ solution for 2 minutes and spread on blotter paper for drying. Leaves were macerated in a small quantity of water by pestle and mortar. Aliquot was considered as standard. One milliliter of each dilution 10^{-1}, 10^{-2} and 10^{-3} made from diseased sample was poured in each separate petri plate by using dilution plate technique (Harris and Sommers, 1968). Plates were allowed to solidify, wrapped and incubated at 30 °C for 72 hours. Infected leaves were surface sterilized in 0.1% $HgCl_2$ solution and rinsed thoroughly with distilled water. Symptomatic leaves were placed on modified nutrient agar plates and incubated at 28 °C. A sterile inoculating loop was rubbed on diseased carpel tissue and streaked on Nutrient agar plates and incubated. In control, asymptomatic diseased tissues were plated as described above. Yellow and round colonies appeared after 96 hours of incubation. Isolated colonies of the bacterium were picked up and single colony was transferred to Nutrient Glucose Agar (NGA) media to produce pure culture (Fig. 1) (Curran *et al.,* 1937). The bacterium was identified on the basis of morphological and biochemical characteristics (Breed *et al.*, 1957). The stock culture of the bacterium was maintained at 4 °C in refrigerator.

Pathogenicity test

The isolated bacterium was tested for its pathogenicity on healthy lime variety Kagzi (*Citrusaurantifolia*). One year old ten citrus plants were purchased from Citrus Research Institute Sargodha. Plants were transplanted in pots containing field soil previously sterilized with Formalin (1:320). The bacterium from stock culture was multiplied on nutrient agar by incubating it for 48 hours at 30 °C. Aqueous suspension of the bacterium having a concentration of approximately 10^8 cells/ ml was prepared by plate count method (Kiraly *et al.,* 1974). Just before inoculation, plants were irrigated and covered with polythene bags for two hours to create a condition of high humidity and placed under sunlight to allow the stomata to open to the maximum (Gunn, 1962).

The abaxial surface of the leaves was inoculated using a spraying machine at a pressure of 1.1 kg/cm² until the tissues showed water soaking. In control the plants were sprayed with distilled water only.

The plants were then kept under observation for two weeks in the greenhouse and symptoms were recorded.

GPS tagging and correlation with environmental parameters with incidence of citrus canker

GPS coordinates were taken using cell phone which had built in navigation system and allows for pin point precision for location detection (Fig. 2). It was helpful to get exact GPS coordinates for latitude and longitudes to dedicate GPS tracker on a map. The GPS coordinates were jotted down into a map application to create a pin point or a spot of that location.

Epidemiological data i.e. minimum and maximum temperatures, wind speed, relative humidity and rainfall were collected after seven days interval from February to end of March from a meteorological station of University of Agriculture, Faisalabad.

Statistical analysis

Statistical analysis was performed through statistical tests by using SAS/ STAT statistical software (SAS Institute, 1990). Data for disease severityand environmental parameters were analyzed through correlation and regression analysis to estimate the relationship with environmental factors and disease progress. Epidemiological factors having statistically significant impact on disease development were graphically plotted.

Management

One-year-old healthy citrus plant were purchased from nursery and transplanted to pots (one plant/pot). These pots were placed in the field in the greenhouse of Department of Plant Pathology, University of Agriculture, and Faisalabad. Providing the conventional agronomic practices for 15 days, six antibiotics Neemosol @ 5ml/L (T_1), Copper hydro oxide @ 3g/L (T_2), Bismerthiazol @ 2g/L (T_3), Streptomycine + Copper oxychloride @ 1 + 3g/L (T_4), Oxytetracycline @ 2g /L (T_5), Kasugamycin @ 3 ml/L (T_6) and one control (T_7) were used at standard dose against *X. a.* pv. *citri.* The experiment was laid out under RCBD with two replications for each treatment (3 pots/ replication). After 24 hours of these treatments, the plants were irrigated and covered with polythene bags for 2 hours to provide artificial conditions of humidity. Aqueous suspension of the bacterium prepared from 48 hours old actively growing culture of *X. a.* pv. *citri* was inoculated with the help of spraying machine having a spraying gun which could produce a pressure of 1.1 kg/cm². The plants inoculated with pathogen only served as a control. Data regarding the disease severity were recorded after 5 days interval (Croxall *et al.,* 1952).For the management of citrus leaf miner (*Phyllocnistic citrella*), six chemicals viz. Feraroron @ 50cc/20L (T_1), Spinosaid @15 ml/20L (T_2), Acetameprid @ 25g/20 L (T_3), Imidacloprid @ 50ml/20L (T_4), Bifenthrin@ 50ml/20L (T_1) and Emamectin benzoate@ 100cc/20L (T_6) were selected and sprayed on the infested citrus plants at standard doses and data was recorded after 24, 48 and 72 hours. In control, sterile distilled water was sprayed as control (T_7). Data was recorded following Johnston & Booth (1983) scale.

Results

GPS incidence of citrus canker and citrus leaf miner

The results of citrus canker and citrus leaf miner population are illustrated in Table 2 and 3, respectively. GPS tagging of all survey locations (Fig. 3) indicated that maximum citrus canker incidence (%) was recorded at Kot Momin mainly, Chak No 21 S.B (32.16078, 73.02801) and Mela (32.16976, 73.13783) while minimum disease was observed in tehsil Sargodha Chak 33 N.B (32.11396, 72.7438) (Table 2).

Table 1. Localities visited in District Sargodha.

Sargodha	Risala No. 5
	Mitha Luck
	Chak 89 N.B Sargodha
	Chak 33 N.B
	Chak No. 96 S.B
	Kudlathi Ara
Sahiwal	Sial Sharif
	Lakhiwal
	Jalla Balla
	Radhan
	Lilyani
Bhalwal	Chak No. 5 N.B
	Chak No. 7 NB
	Chak No. 9 Nb
	Chak No. 18 N.B
	Chak NO. 111 N.B
Sillan wali	Chak NO. 48 S.B.
	Chak No. 127 N.B.
	Chak No. 135 S.B.
	Chak No. 118 N.B.
	Chak Kot Bhai Khan
Shahpur	Khan Pur
	Sultan Pur
	Allahbad
	Jallal Pur
	Chak 21 S. B
Kot Momin	Midh Ranjha
	Mela
	Matella
	Hujjan

Similarly, maximum citrus leaf miner incidence was recorded in Bhalwal Lilyani(32.21312, 72.94722) whereas, Risala No. 5 (32.11998, 72.67975) andMitha Luck (32.13343, 72.77768) localities of Sargodha were found less effectedwith this incidence (Table 3).

Table 2. Incidence of citrus canker in different tehsils of Sargodha.

Sr. No.	Sargodha	GPS Locations	Disease incidence of randomly selected 5 plants in each orchard			
			1	2	3	Mean
1	Risala No. 5	(32.11998, 72.67975)	17	16	22	18.33
2	Mitha Luck	(32.13343, 72.77768)	19.5	20	18	19.17
3	Chak 89 N.B Sargodha	(32.02687, 72.61593)	15	20.5	19	18.17
4	Chak 33 N.B	(32.11396, 72.7438)	14	15.5	15.5	15
5	Chak No. 96 S.B	(32.01146, 72.75807)	18	18	17.5	17.83
	Sahiwal					
1	Kudlathi Ara	(32.01558, 72.39201)	18	19	17	18
2	Sial Sharif	(31.90769, 72.29235)	19	20.5	22	20.5
3	Lakhiwal	(31.97193, 72.3063)	19.5	16	18.5	18
4	Jalla Balla	(31.9924, 72.4019)	18	16.5	17	17.17
5	Radhan	(31.90874, 72.43294)	21	16.5	18.5	18.67
	Bhalwal					
1	Lilyani	(32.21312, 72.94722)	19	19.5	18	18.83
2	Chak No. 5 N.B	(32.28288, 72.92791)	14.5	18	19.5	17.33
3	Chak No. 7 NB	(32.29491, 72.89909)	19.5	20	20	19.83
4	Chak No. 9 Nb	(32.23529, 72.88407)	20	20	17.5	19.17
5	Chak No. 18 N.B	(32.2602, 72.81836)	18.5	18.5	19.5	18.83
	Sillan wali					
1	Chak NO. 111 N.B	(31.94943, 72.57094)	20	19	20.5	19.83
2	Chak NO. 48 S.B.	(31.85403, 72.70794)	16	16	19	17
3	Chak No. 127 N.B.	(31.88087, 72.63364)	20.5	20.5	17.5	19.5
4	Chak No. 135 S.B.	(31.8417, 72.58383)	17.5	18.5	20	18.67
5	Chak No. 118 N.B.	(31.90754, 72.53639)	16.5	19	17.5	17.67
	ShahPur					
1	Chak Kot Bhai Khan	(32.35331, 72.58104)	16.5	18	18.5	17.67
2	Khan Pur	(32.25001, 72.62475)	19.5	20.5	19.5	19.83
3	Sultan Pur	(32.12209, 72.39919)	14.5	17	19.5	17
4	Allahbad	(32.18102, 72.43603)	14.5	16.5	18	16.33
5	Jallal Pur	(32.29247, 72.4448)	17.5	18.5	18.5	18.17
	Kot Momin					
1	Chak 21 S. B	(32.16078, 73.02801)	22	20	18	20
2	Midh Ranjha	(32.05962, 73.17903)	18.5	19.5	18.5	18.83
3	Mela	(32.16976, 73.13783)	20.5	21.5	19	20.33
4	Matella	(32.09335, 72.95748)	19	18	18	18.33
5	Hujjan	(32.2688, 73.11547)	20	20	19	19.67

Table 3. Incidence of citrus Leaf Miner in different tehsils of Sargodha.

Sr. No.	Sargodha	GPS Location	Disease incidence of randomly selected from 5 plants in each orchard			
			1	2	3	Mean
1	Risala No. 5	(32.11998, 72.67975)	1.8	1	1.6	1.47
2	Mitha Luck	(32.13343, 72.77768)	1.6	1.4	1.4	1.47
3	Chak 89 N.B Sargodha	(32.02687, 72.61593)	2.6	1.2	2.2	2
4	Chak 33 N.B	(32.11396, 72.7438)	1.8	2	2.6	2.13

5	Chak No. 96 S.B	(32.01146, 72.75807)	1.4	2	2	1.8
	Sahiwal					
1	Kudlathi Ara	(32.01558, 72.39201)	1.6	1.4	2.75	1.97
2	Sial Sharif	(31.90769, 72.29235)	2.6	2.6	4	3.07
3	Lakhiwal	(31.97193, 72.3063)	2.8	3	3.4	3.07
4	Jalla Balla	(31.9924, 72.4019)	1.4	2.4	1.2	1.67
5	Radhan	(31.90874, 72.43294)	1.4	2.6	2.4	2.13
	Bhalwal					
1	Lilyani	(32.21312, 72.94722)	2.8	3.4	1.8	2.67
2	Chak No. 5 N.B	(32.28288, 72.92791)	2	2.2	3	2.4
3	Chak No. 7 NB	(32.29491, 72.89909)	2.75	1.4	2.4	2.13
4	Chak No. 9 Nb	(32.23529, 72.88407)	3.6	2.2	2	2.6
5	Chak No. 18 N.B	(32.2602, 72.81836)	2.4	2.6	1.4	2.13
	Sillan wali					
1	Chak NO. 111 N.B	(31.94943, 72.57094)	3.25	2.6	2.2	2.63
2	Chak NO. 48 S.B.	(31.85403, 72.70794)	1.8	1.8	1	1.53
3	Chak No. 127 N.B.	(31.88087, 72.63364)	2.4	3	3	2.8
4	Chak No. 135 S.B.	(31.8417, 72.58383)	2.6	2.2	0.6	1.8
5	Chak No. 118 N.B.	(31.90754, 72.53639)	2.2	1.6	1.8	1.87
	ShahPur					
1	Chak Kot Bhai Khan	(32.35331, 72.58104)	2.2	1.6	2	1.93
2	Khan Pur	(32.25001, 72.62475)	1.8	2.2	1.6	1.87
3	Sultan Pur	(32.12209, 72.39919)	1.4	2.4	1.6	1.8
4	Allahbad	(32.18102, 72.43603)	1.6	2.4	2	2
5	Jallal Pur	(32.29247, 72.4448)	2	2	1.8	1.93
	Kot Momin					
1	Chak 21 S. B	(32.16078, 73.02801)	1.2	2.4	2.2	1.93
2	Midh Ranjha	(32.05962, 73.17903)	2.6	2	1.8	2.13
3	Mela	(32.16976, 73.13783)	2.2	2.4	2.4	2.33
4	Matella	(32.09335, 72.95748)	2.8	1.6	2.6	2.33
5	Hujjan	(32.2688, 73.11547)	2	2.2	2.4	2.2

Correlation of environmental conditions with citrus canker disease incidence and citrus leaf miner populations

Correlations of environmental conditions with citrus canker and white fly populations were observed at all six locations. Except relative humidity all environmental factors i.e. minimum and maximum temperatures, rainfall and wind speed demonstrated statistically significant correlation with citrus canker incidence (Table 4) and citrus leaf miner population (Table 5) at all locations.

Table 4. Correlation of environmental factors with citrus canker disease incidence.

Locations	Maxi. Temp. (°C)	Mini. Temp. (°C)	Relative Humidity (%)	Rainfall (mm)	Wind speed (km/ha)
Sargodha	0.5335*	0.4800*	-0.3391*	0.4864*	0.6075*
	0.0345	0.0143	0.0556	0.0046	0.0277
Sahiwal	0.3107*	0.3417*	-0.2520*	0.2318*	0.1864*
	0.0168	0.0537	0.0268	0.0299	0.0467
Bhalwal	0.3527*	0.3527*	-0.3165*	0.3165*	0.3926*

	0.0456	0.0456	0.0369	0.0369	0.0135
Sillan Wali	0.2938*	0.3518*	-0.3518*	0.5614*	0.5318*
	0.0313	0.0514	0.0416	0.0315	0.0156
Shahpur	0.3369*	0.3330*	-0.2972*	0.2972*	0.9227*
	0.0579	0.0485	0.0273	0.0273	0.0372
Kot Momin	0.1858*	0.2755*	-0.2223*	0.7194*	0.2223*
	0.0467	0.0356	0.0194	0.0223	0.0194

Upper values indicate Pearson's correlation coefficient; Lower values indicate significance at 5% level of probability.

Table 5. Correlation of environmental factors with citrus leaf miner population.

Locations	Maxi. Temp. (°C)	Mini Temp. (°C)	Relative Humidity (%)	Rainfall (mm)	Wind speed (km/ha)
Sargodha	0.6776*	0.6249*	-0.6129*	0.6629*	0.7525*
	0.0287	0.0259	0.0217	0.0222	0.0142
Sahiwal	0.3171*	0.2196*	-0.2326*	0.1399*	0.6998*
	0.0361	0.0227	0.0567	0.0225	0.0283
Bhalwal	0.5338*	0.5383*	-0.5726*	0.7256*	0.4917*
	0.0342	0.0345	0.0313	0.0133	0.0042
Sillan Wali	0.3363*	0.3806*	-0.3806*	0.8360*	0.5273*
	0.0058	0.0273	0.0537	0.0372	0.0386
Shahpur	0.3369*	0.1639*	-0.2774*	0.6516*	0.2774*
	0.0357	0.0237	0.0156	0.0274	0.0561
Kot Momin	0.6381*	0.5510*	-0.6985*	0.8915*	0.6985*
	0.0246	0.0358	0.0189	0.0589	0.0189

Upper values indicate Pearson's correlation coefficient; Lower values indicate significance at 5% level of probability.

Table 6. Analysis of variance for the evaluation of chemicals for the management of citrus canker disease severity.

Source of Variation	Degree of Freedom	Sum of Square	Mean Sum of Square	F-value	P-value
Treatments	6	1936.51	322.752	123.48	0.0005**
Replications	4	12.04	3.01		
Error	24	62.73	2.614		
Total	34	2011.28			

Characterization of environmental factors conducive for the development of Xanthomonas axonopodis pv. citri and citrus leaf miner

Citrus canker disease incidence and citrus leaf minor data of all locations were subjected to regression analysis to characterize the critical ranges of epidemiological conditions effective for disease development. Results indicated that maximum disease incidence was recorded in tehsil Kot Momin at maximum and minimum temperature 41°C, 27 °C, rainfall 33 mm and wind speed 7 km/h; while all other localities showed less disease incidence at same environmental conditions Fig.4 to 7. Similarly,maximum whitefly population was observed in tehsil Sahiwal at maximum temperature 39 °C, minimum temperature 23 °C, rainfall 39 mm and wind speed 8 km/h (Fig. 8to 11). A decreasing trend in citrus canker and citrus leaf miner population with relative humidity was observed in all six localities i.e. single units increase in relative humidity both incidence of citrus canker and CLM population decreased (Fig. 12 to 13).

Table 7. Response of different plant Extracts and chemicals against canker disease management.

Chemicals	Disease Severity (%)
Neemosol @ 5ml/L	13.00D
Copper hydro oxide @ 3g/L @ 3g/L	22.80B
Bismerthiazol @ 2g/L	17.00C
Streptomycine + Copper oxychloride @ 1 +3g/L @ 1 + 3g/L	06.80E
Oxytetracycline @ 2g /L	12.20D
Kasugamycin @ 3 ml/L	11.01D
LSD= 2.11	

Values sharing same letters do not differ from each other significantly.

Table 8. Analysis of variance of evaluation of chemicals for the management of citrus leaf miner population at 24 hours of pre-treatment.

Source of Variation	Degree of Freedom	Sum of Square	Mean Sum of Square	F-value	P-value
Treatments	7	3607.80	515.40	634.39**	0.00
Replications	2	1.94	0.972		
Error	14	11.37	0.812		
Total	23	3621.12			

Grand Mean: 28.38; CV 3.18%; ** = Significant at $P \leq 0.05$

Table 9. Analysis of variance of evaluation of chemicals for the management of citrus leaf miner population at 24 hours of post-treatment.

Source of Variation	Degree of Freedom	Sum of Square	Mean Sum of Square	F-value	P-value
Treatments	7	4475.64	639.37	50.17**	0.00
Replications	2	24.7	12.38		
Error	14	178.41	12.74		
Total	23	4678.83			

Grand Mean: 44.7; CV: 7.98%; ** = Significant at $P \leq 0.05$.

Management

Analysis of variance indicated that all chemicals showed significant impact individually in reducing the citrus canker disease severity as compared to control at 5% level of probability (Table 6). By comparing the action of individual chemicals, Streptomycine + Copper oxychloride @ 1 + 3g/L was found most effective (6.8%) whereas copper hydroxide was least effective (22.8%) in reducing disease severity as compared to control (30.4%) (Table 7).

Table 10. Analysis of variance of evaluation of chemicals for the management of citrus leaf miner population at 48 hours of post-treatment.

Source of Variation	Degree of Freedom	Sum of Square	Mean Sum of Square	F-value	P-value
Treatments	7	6505.34	929.33	10.03**	0.00
Replications	2	182.57	91.28		
Error	14	1296.61	92.61		
Total	23	7984.53			

Grand Mean: 76.46; CV: 12.59%; ** = Significant at $P \leq 0.05$.

All chemicals showed significant impact for reducing the citrus leaf miner population as compared to control after 24, 48 and 72 hours, respectively at 5% level of significance (Table 8-11). Comparison of each chemicals indicated that Match gave maximum reduction in citrus leaf miner population with 33.62, 27.29 and 20.10% infestation after 24, 48 and 72 hours of application respectively. While Emamectin benzoate was least effective in reducing the citrus leaf miner population exhibiting 45.42, 37.35 and 31.61% infestation after 24, 48 and 72 hours, respectively as compared to control (Fig. 14).

Table 11. Analysis of variance of evaluation of chemicals for the management of citrus leaf miner population at 72 hours of post-treatment.

Source of Variation	Degree of Freedom	Sum of Square	Mean Sum of Square	F-value	P-value
Treatments	7	5424.87	77442.46.98	17.90**	0.00
Replications	2	84.92	45.46		
Error	14	606.26	43.30		
Total	23	6116.05			

Grand Mean: 66.08; CV: 9.96%; ** = Significant at $P \leq 0.05$.

Discussion

GPS incidence of citrus canker and citrus leaf miner

A comprehensive survey study for citrus canker and leaf miner incidence was conducted in six tehsils of district Sargodha.

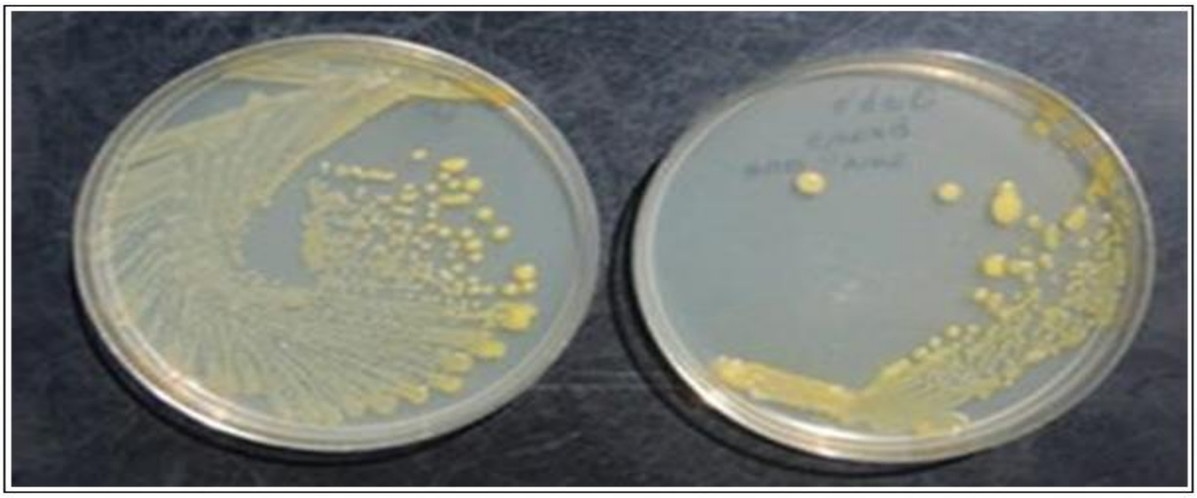

Fig. 1. Pure Culture of *Xanthomonas axonopodis* pv. *citri.*

Fig. 2. *Taking GPS Coordinates.*

Results showed that maximum citrus canker (73.13%) and leaf miner (72.94%) incidence was recorded in Kot Momin and Bhalwal, respectively. Similarly, Derso and Sijam (2007) conducted surveys in Ethiopia and indicated that overall incidence of disease on leaves was 71.4%. Morphological, biochemical and physiological determination of characteristics and leaf isolation tests clearly determined the presence of *Xac*. In Ethopia, Xac was found on Mexican lime (*C.aurantifolia*) and sour orange (*C. aurantium*). Considering the hosts and isolated leaf assays, itwas found that Xac isolate in Ethiopia showed similarities with typical Asiatic form (*Xac*-A).

Fig. 3. GPS Tagging of the Locations.

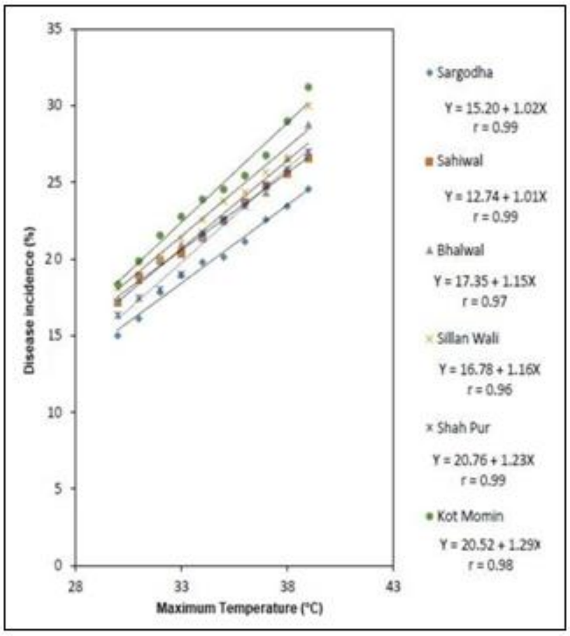

Fig. 4. Effect of maximum temperature on citrus canker disease incidence in various tehsils of Sargodha district during 2016

Canker incidence showed a significant correlation with temperature fluctuations but showed a non-significant correlation with rainfall, altitude or age of plant. Latif and Younis (1961) determined that the CLM was active and reproduced on newly developed seedlings throughout the year.

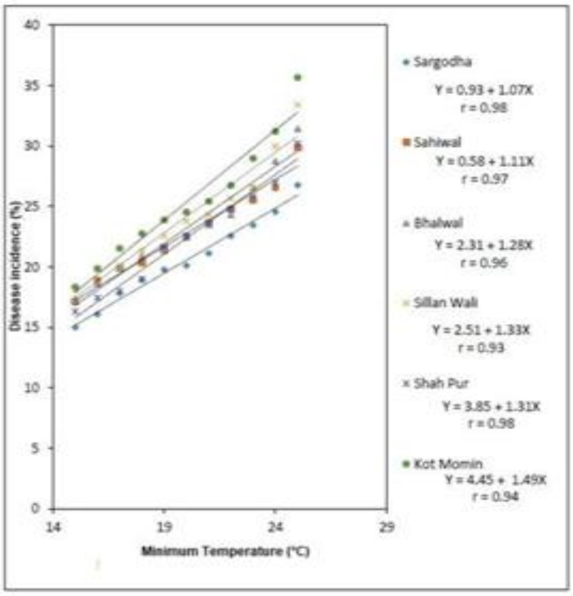

Fig. 5. Effect of minimum temperature on citrus canker disease incidence in various tehsils of Sargodha district during 2016.

The duration of various stages of reproduction depended upon the prevailing temperature. In late spring or summer, the development of CLM was five or six times faster as compared to autumn or early spring. All stages of life cycle occurred throughout the year except in January and February when only the larval and pupal stages were present.

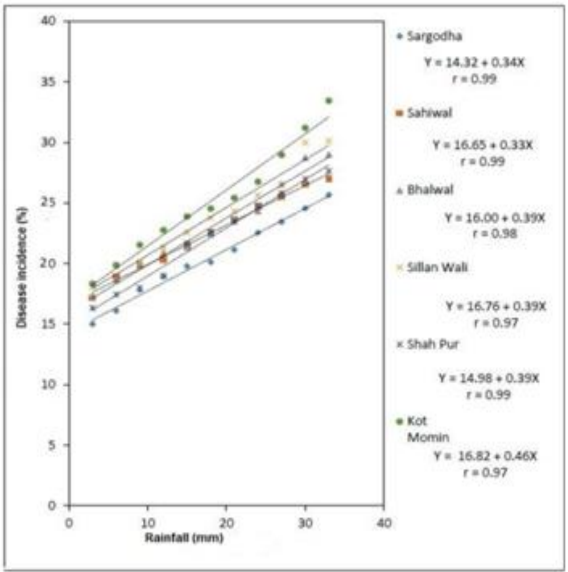

Fig. 6. Effect of rainfall on citrus canker disease incidence in various tehsils of Sargodha district during 2016.

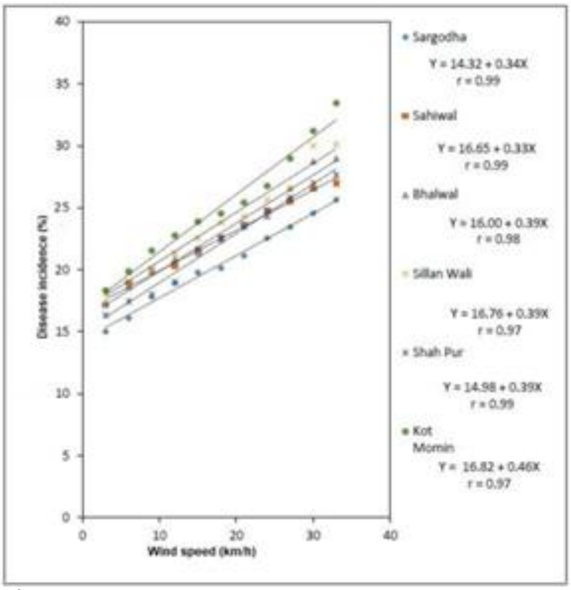

Fig. 7. Effect of wind speed on citrus canker disease incidence in various tehsils of Sargodha district during 2016.

Its population was maximum first during Months of March-May and then again from September-October under suitable environmental conditions. The temperature and relative humidity in spring and autumn seasons showed a great impact on the CLM infestation and increased plant damage. During winter, persistence of low temperature and during monsoon season the appearance of high rainfall adversely affects the CLM population Khan *et al.* (2000).

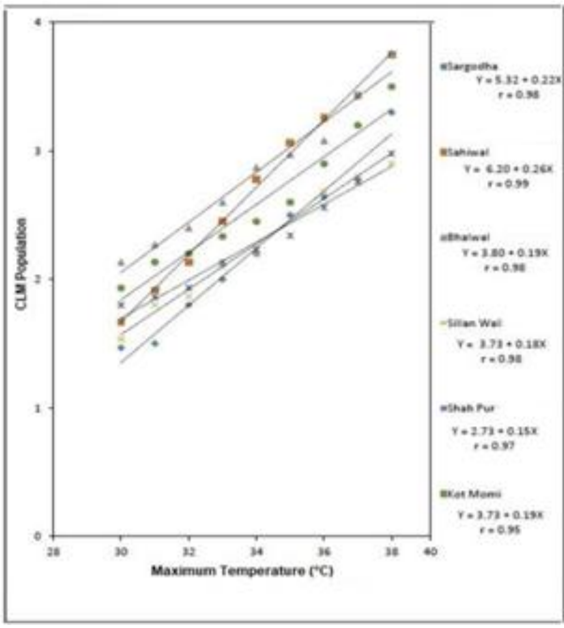

Fig. 8. Effect of maximum temperature on citrus leaf miner population in various Tehsils of Sargodha district during 2016.

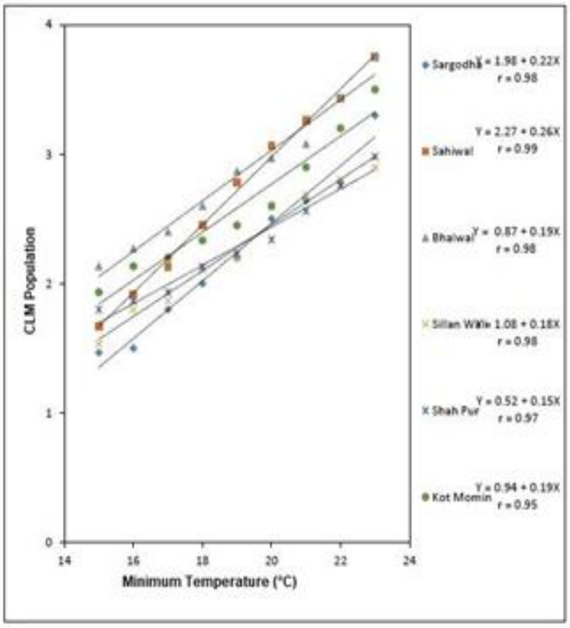

Fig. 9. Effect of minimum temperature on citrus leaf miner population in various Tehsils of Sargodha district during 2016.

Characterization of environmental conditions conducive for citrus canker and citrus leaf miner populations

Considering the correlation of environmental factors with citrus canker and citrus leaf minor it was found that all environmental factors had significant correlation with disease severity and CLM population.

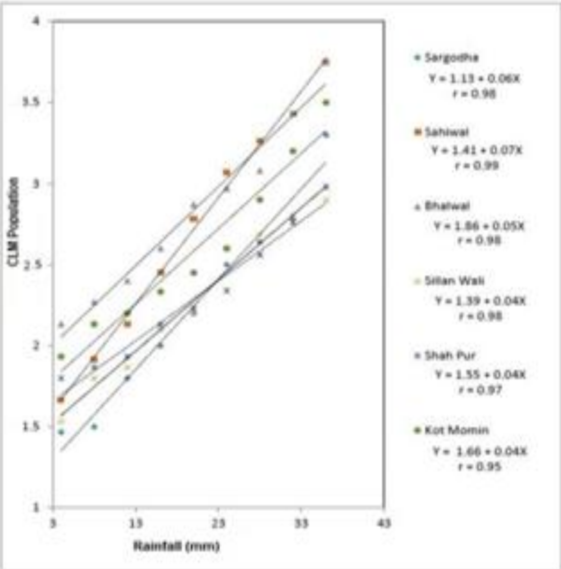

Fig. 10. Effect of rainfall on citrus leaf miner population in various tehsils of Sargodha district during 2016.

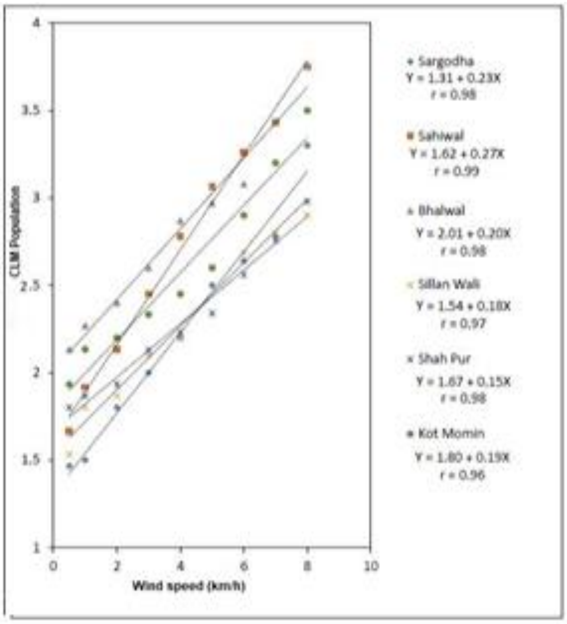

Fig. 11. Effect of wind speed on citrus leaf miner population in various tehsils of Sargodha district during 2016.

These results are in conformation with the investigation of Graham *et al.* (2004) who concluded that the optimum temperature range for growth of bacterium is 28-39 °C with maximum temperature ranging from 35-39 °C. Ramkrishana (1954) determined that unrestricted wetness on the host superficial layer for a period of 20 minutes found necessary for successful entry of bacterium.

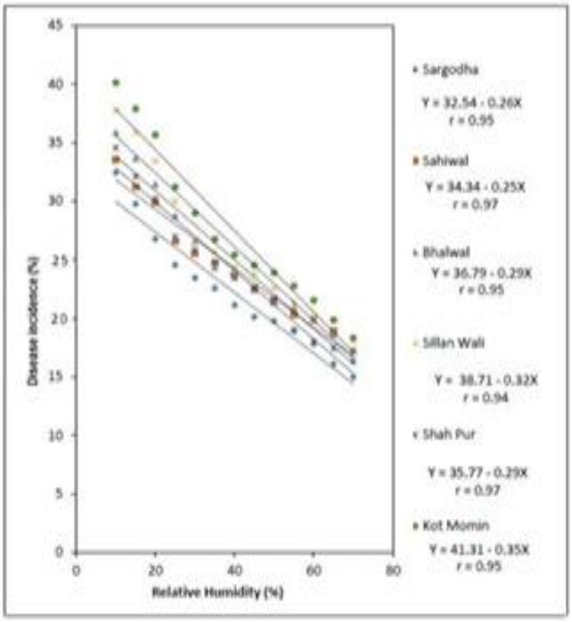

Fig. 12. Effect of relative humidity on citrus canker disease incidence in various tehsils of Sargodha district during 2016.

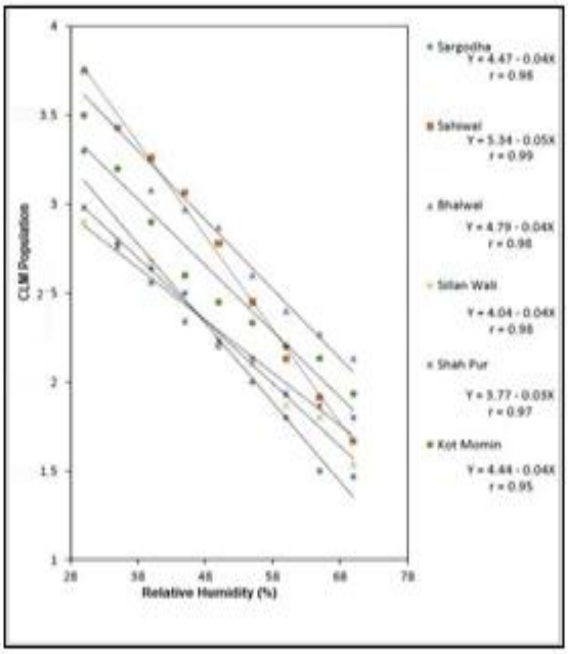

Fig. 13. Effect of relative humidity on citrus leaf miner population in various tehsils of Sargodha district during 2016.

The disease occurs in severe form when wind speed exceeds by 8 m/s (Kuhara, 1978) and pathogen move a distance of 11.2 km from one region to other Gottwald *et al.* (2001). Similarly, Srivastava *et al.* (1997) conducted field trials to study the epidemiology of *Xac*. The highest disease intensities over two seasons were recorded in the month of September when the average temperature, relative humidity and rainfall were 29-29.4 °C, 80-95.5 % and 8.92-9.97 mm, respectively. With a decrease in temperature, rainfall and relative humidity, there was considerable reduction of disease intensity by the end of September.

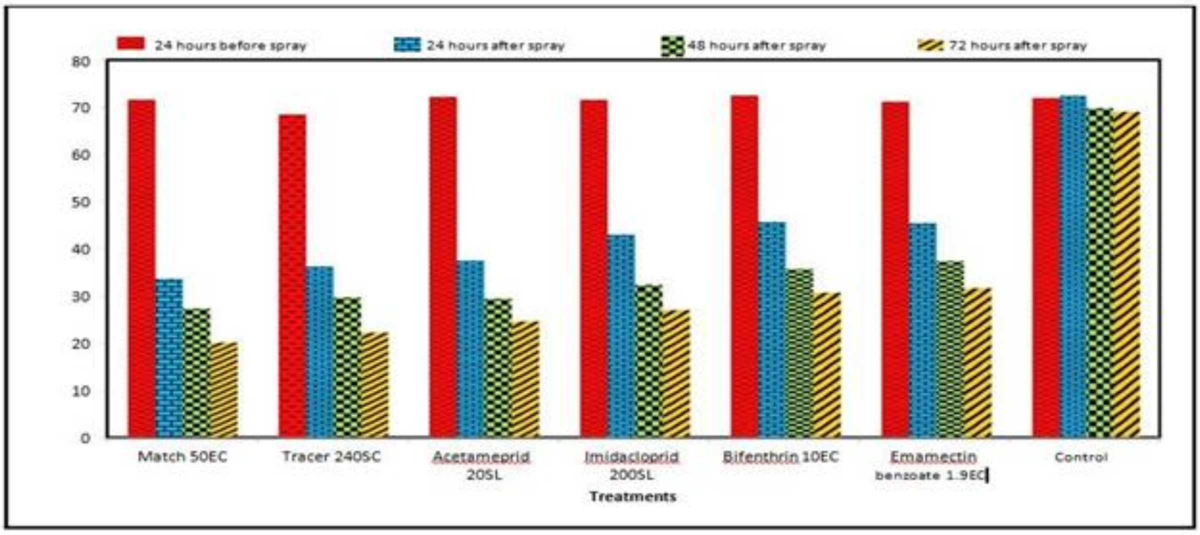

Fig. 14. Response of different chemicals against CLM population management.

Management strategies used for the management of citrus canker and citrus leaf miner populations

On the basis of environmental variables, chemical applications were tested against *Xac* and citrus leaf miner. It was confirmed that streptomycine + copper oxychloride showed effective results against *Xac* severity. These results showed clear similarity with the investigation of Kale *etal.* (1994) who found that streptomycin and $CuSO_4$ in addition to aureofungin solutions gave good control of citrus canker. Disease was completely controlled with copper oxychloride @ 0.3%, Mancozeb @ 0.2 % and streptomycin + oxytetracycline @ 0.005% on spray after 15 days interval from early July to pruning of the infected leaves and twigs. The Cu $(OH)_2$ formulation @ 1g/2 liter gave effective control of *Xac* and inorganic copper formulations ($Cu(OH)_2$ and $CuSO_4$) were not phytotoxic to young leaves of natsudaidai and Satsuma leaves (Alditar 1999). The results of citrus leaf miner management showed strong resemblance with Pervoc *et al.* (2006) in which neonicotinoid and avermectin based insecticides were found most effective in controlling CLM. An investigation was conducted to compare the efficacy of different insecticides and neem containing botanical formulations against CLM. Neem formulations containing neem seed 65 kernel extract and active ingredient Azadirachtin showed high mortality of insect (Jayanthi and Verghese, 2004). Alcohol based extracts of 26 non-host species and a horticultural mineral oil (HMO) was evaluated against *P. citrella* and it was found that Mikania micrantha, Ageratum conyzoides and Dicranopteris pedata showed more than 90% preventive effects on oviposition rate of insect (Xiao *et al.*, 2007).

Conclusion

It was concluded that maximum disease incidence and citrus leaf minerpopulation wererecorded in Kot Momin and Bhalwal mainly in Lilyani(32.21312, 72.94722)respectively, among all tehsils of district Sargodha. Except relative humidity,all epidemiological variables showed significant correlationwith both disease incidence and citrus leaf miner population at all six localities. Streptomycine + Copper oxychloride match indicated most effective control against citrus canker and citrus leaf miner population as compared to control.

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