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EPIDEMIOLOGICAL MARKERS FOR CITRUS CANKER CAUSED BY *XANTHOMONAS CITRI* PV. *CITRI*

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ABSTRACT

Climatic factors have a significant impact on the growth and development of citrus canker disease, which is one of the biggest threats to the citrus industry caused by the bacterium *Xanthomonas citri* pv. *citri (Xcc).* The growth stage, host sensitivity, succulence, vigour, survival, rate of multiplication, pathogen dispersal direction, rate of spore penetration, and germination are all impacted by these factors. Climatic factors such as maximum and minimum temperatures, relative humidity, rainfall, and wind speed were studied in the experiment in order to determine their effects on the development of disease. Significant positive association was observed on ten varieties/cultivars (Grapefruit, Rough lemon, Kinnow, Red blood, Shamber, Duncan, Foster, Malta, Citrus Sinensis and China lemon). To find the correlation between climatic factors and disease projection, a multiple regression model based on a two-year study was developed. The goodness of model was signified by coefficient determination value. There was a significant positive association among all ten varieties. It was concluded that all the climatic factors like max. T (37 ^{0}C), min. T (27 ^{0}C), RH 55%, RF (4.7-7.1 mm) and WS 8 Km/h were the conditions for the development of canker disease. This study would be beneficial for researchers to develop better disease management strategies for the future as a result of changing climatic conditions against disease.

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INTRODUCTION

Citrus is one of the world's major fruit crops, belonging to the family *Rutaceae*. The most renowned species of citrus with commercial significance are oranges (*Citrus sinensis*), lemons (*Citrus limon*), limes (*Citrus aurantiifolia*), grapefruits (*Citrus paradisi*) and mandarins (*Citrus reticulata*). Citrus is grown in more than 130 countries. Its origin is probably temperate and subtropical areas of Southeast Asia. Due to its high production it stands first among all fruits in the whole world (Ladaniya, 2010). In Pakistan, production rate is 2.2 million tonnes and categorized 12th among all the countries of world (Memon and Kasbit, 2017). Citrus is damaged by a number of destructive diseases like Citrus Huanglongbing, canker, gummosis, scabs, decline and brown rot. Among all these diseases bacterial citrus canker is the most devastating disease caused by *Xanthomonas citri*pv. *citri (Xcc)*. There are many distinctive kinds of citrus canker (canker A, cancrosis B, cancrosis C and cancrosis D) caused by many pathovars of *Xanthomonas.* The Asiatic type of canker (canker A) is most severe and prevalent form of canker caused by the Asian strain *Xanthomonas citri* (Cubero and Graham, 2002). After gaining entrance into leaves, the bacteria established and causes infection following a series of highly coordinated events; first of all, the bacteria gain entrance into the leaves and occupies the intercellular spaces, then it uses type III secretion attach to the mesophyll cells (Kumar *et al.*, 2011). *Xcc* is a gram negative, straight rod shape, mono-flagellum bacteria which causes early fruit drop, drying of twigs, and leaf fall, which results in low-quality fruits and yield losses (Gottwald *et al.*, 2002). Environment plays a crucial part in multiplication and dispersion of *Xcc*. Among epidemiological factors temperature (30-38 °C) with high relative humidity (RH) plays important role (Graham *et al.*, 2004) but Agrios (2005) and Khan and Abid (2007) observed that temperature (max. and min.) along with wind velocity, RH and rainfall (RF) had principle role in the citrus canker. Further it was also noted that splashes of rain resulting from heavy winds provide favourable conditions for the spread of CC (Yan and Wang, 2012). The susceptibility of host, survival, vigour, succulence, penetration rate of spores, rate of multiplication, direction of pathogen dispersion, and germination are all impacted by these variables (Chakraborty and Newton, 2011). The pathogen becomes more aggressive in causing infection and transmission during the hot summer and mild winter with some variations. With the raindrop splashes, *Xcc* spread to new healthier host plants and develop lesions on the surface of leaves, stems, and fruits. The rate of bacteria spread to infect the new host plant increased in the presence of free moisture. CC epidemics are increased during monsoon rainstorms accomplished with strong winds when an active source of inoculum is present (Graham *et al.*, 2004).

Pakistan has eight different climatic zones in which climate change every year than the previous one (Bhutta, 2006). From the last several years, Climate changes are resulting unpredictable plant development conditions, which is subsequently exerting a significant amount of strain on production of food. The study of epidemiological variables benefits in strategies of management by paying attention to the climatic factors that favor the development of CC disease (Singh and Thind, 2014). Currently, a very little work has been done on characterization of epidemiological factors in Pakistan especially in citrus growing regions and a proper understanding of epidemiology factors is necessary for effectual management of disease. So, role played by environmental parameters under the local climatic conditions, citrus canker spreads and persists on various varieties was done.

MATERIAL AND METHODS

Data Collection

A study was designed for two years on the influence of environmental variables, including max. and min. T (^{0}C), WS (Km/h), RF (mm), and RH (%) in the development of Citrus canker disease on ten citrus varieties (Grapefruit, Rough lemon, Kinnow, Red blood, Shamber, Duncan, Foster, Malta, Citrus Sinensis and China lemon). Environmental parameters were obtained from a meteorological station UAF, and their compatibility with the disease development on the ten citrus varieties was examined using regression analysis and focusing on results a predictive model was developed.

Regression Analysis

Multiple regression analysis was used to determine the association between climatic and environmental parameters and disease incidence and development (Chatterjee and Hadi, 2013). Multiple linear regression (MLR) models include multiple explanatory or predictor variables (X) (Eq.1). The following equation represents the relationship between the variables in the multiple regression model:

$$Y = \beta 0 + \beta 1x1 + \beta 2x2 + \cdots + \beta i\, xi + \in \cdots \ldots \ldots \ldots \text{(Eq.1)}$$

By using multiple regression models to compare the observed and predicted disease incidence values, the impact of these factors on the development of CC was studied. Additionally, environmental factors that have a huge influence on the growth of CC disease were taken when developing disease predicting models.

All data pertaining to disease incidence and environmental factors is appended as supplementary file to this paper online.

RESULTS

Analysis of variance of regression articulated that T (max. & min.), RH, RF and WS have significant contributions towards development of disease. The R_2 value 97.5 expressed the model to be statistically suitable under the given environmental conditions.

Multiple Regression Models and Scattered Plot on Citrus Varieties/Cultivars

The multiple regression equation of citrus canker predictive models is shown in table 1 for the first year and table 2 for the second year. Here Y = Disease incidence X_1 = Max. T, X_2 = Min. T, X_3= RH, X_4 = RF and X_5 = WS). The R_2 values expressed that all the models were statistically appropriate under prescribed environmental conditions.

Table 1. Multiple regression equations of ten varieties based on weekly environmental conditions and predicted citrus canker incidence for 1st year.

Sr.#	Multiple Regression equation	R-Sq
1	$Y_1 = 31.2 + 0.934 X_1 + 0.159 X_2 + 0.380 X_3 + 3.83 X_4 + 2.45 X_5$ Y_1 = Grapefruit; X_1 = Max. temperature (°C) X_2 = Min. temperature (°C); X_3 = Relative humidity (%) X_4 = Rainfall (mm); X_5 = Wind speed (Km/h)	98.39
2	$Y_2 = 20.0 + 0.840 X_1 + 0.208 X_2 + 0.150 X_3 + 2.65 X_4 + 0.27 X_5$ Y_2 = Rough lemon; X_1 = Max. temperature (°C) X_2 = Min. temperature (°C); X_3 = Relative humidity (%) X_4 = Rainfall (mm); X_5 = Wind speed (Km/h)	98.67
3	$Y_3 = 2.2 + 0.514 X_1 + 0.3542 X_2 + 0.091 X_3 + 3.477 X_4 + 0.187 X_5$ Y_3 = Kinnow; X_1 = Max. temperature (°C) X_2 = Min. temperature (°C); X_3 = Relative humidity (%) X_4 = Rainfall (mm); X_5 = Wind speed (Km/h)	99.04
4	$Y_4 = 3.7 + 0.421 X_1 + 0.246 X_2 + 0.038 X_3 + 5.27 X_4 + 1.69 X_5$ Y_4 = Red blood; X_1 = Max. temperature (°C) X_2 = Min. temperature (°C); X_3 = Relative humidity (%) X_4 = Rainfall (mm); X_5 = Wind speed (Km/h)	98.41
5	$Y_5 = 36.4 + 0.463 X_1 + 0.314 X_2 + 0.911 X_3 + 5.15 X_4 + 2.47 X_5$ Y_5 = Shamber; X_1 = Max. temperature (°C) X_2 = Min. temperature (°C); X_3 = Relative humidity (%) X_4 = Rainfall (mm); X_5 = Wind speed (Km/h)	98.55
6	$Y_6 = 3.9 + 0.164 X_1 + 0.3178 X_2 + 0.084 X_3 + 3.52 X_4 + 0.99 X_5$ Y_6 = Duncan; X_1 = Max. temperature (°C) X_2 = Min. temperature (°C); X_3 = Relative humidity (%) X_4 = Rainfall (mm); X_5 = Wind speed (Km/h)	98.86
7	$Y_7 = 26.3 + 0.417 X_1 + 0.397 X_2 + 0.426 X_3 + 3.10 X_4 + 1.32 X_5$ Y_7 = Foster; X_1 = Max. temperature (°C) X_2 = Min. temperature (°C); X_3 = Relative humidity (%) X_4 = Rainfall (mm); X_5 = Wind speed (Km/h)	97.99
8	$Y_8 = 0.7 + 0.847 X_1 + 0.133 X_2 + 0.279 X_3 + 3.93 X_4 + 0.40 X_5$ Y_8 = Malta; X_1 = Max. temperature (°C) X_2 = Min. temperature (°C); X_3 = Relative humidity (%) X_4 = Rainfall (mm); X_5 = Wind speed (Km/h)	98.35
9	$Y_9 = 2.5 + 0.845 X_1 + 0.168 X_2 + 0.313 X_3 + 3.58 X_4 + 0.58 X_5$ Y_9 = Citrus sinensis; X_1 = Max. temperature (°C) X_2 = Min. temperature (°C); X_3 = Relative humidity (%) X_4 = Rainfall (mm); X_5 = Wind speed (Km/h)	98.34
10	$Y_{10} = 1.5 + 0.999 X_1 + 0.061 X_2 + 0.276 X_3 + 4.76 X_4 + 0.46 X_5$ Y_{10} = China lemon; X_1 = Max. temperature (°C) X_2 = Min. temperature (°C); X_3 = Relative humidity (%) X_4 = Rainfall (mm); X_5 = Wind speed (Km/h)	98.35

Scatter plots of ten varieties (Grapefruit, Rough lemon, Kinnow, Red blood, Shamber, Duncan, Foster, Malta, Citrus sinensis, China lemon) expressed relationship of multiple environmental variable alongside disease incidence and these were subjected for regression for developing models of multiple regression and values of disease incidence which were observed and predicted were in close similarity with ten varieties in 1st year (Figure 1 and 2) and second year respectively (Figure 3 and 4).

The value of R^2 for the following varieties was i.e. Grapefruit (98.39), Rough lemon (98.67), Kinnow (99.04), Red blood (98.41), Shamber (98.55), Duncan (98.86), Foster (97.99), Malta (98.35), Citrus sinensis (98.34) and China lemon (98.35) during first year while 98.03, 98.30, 97.57, 97.78, 98.36, 98.92, 97.53, 98.47, 98.63 and 98.08 during second year consistently.

Table 2. Multiple regression equations of ten varieties based on weekly environmental conditions and predicted citrus canker incidence for 2nd year.

Sr.#	Multiple Regression equations	R-Sq
1	$Y_1 = 54.7 + 0.473 X_1 + 0.521 X_2 + 1.268 X_3 + 0.14 X_4 + 15.94 X_5$ Y_1 = Grapefruit; X_1 = Max. temperature (°C) X_2 = Min. temperature (°C); X_3 = Relative humidity (%) X_4 = Rainfall (mm); X_5 = Wind speed (Km/h)	98.03
2	$Y_2 = 17.8 + 0.864 X_1 + 0.208 X_2 + 0.644 X_3 + 0.49 X_4 + 10.19 X_5$ Y_2 = Rough lemon; X_1 = Max. temperature (°C) X_2 = Min. temperature (°C); X_3 = Relative humidity (%) X_4 = Rainfall (mm); X_5 = Wind speed (Km/h)	98.30
3	$Y_3 = 40.8 + 0.693 X_1 + 0.466 X_2 + 1.085 X_3 + 1.67 X_4 + 15.6 X_5$ Y_3 = Kinnow; X_1 = Max. temperature (°C) X_2 = Min. temperature (°C); X_3 = Relative humidity (%) X_4 = Rainfall (mm); X_5 = Wind speed (Km/h)	97.57
4	$Y_4 = 32.9 + 0.598 X_1 + 0.487 X_2 + 0.903 X_3 + 7.62 X_4 + 22.47 X_5$ Y_4 = Red blood; X_1 = Max. temperature (°C) X_2 = Min. temperature (°C); X_3 = Relative humidity (%) X_4 = Rainfall (mm); X_5 = Wind speed (Km/h)	97.78
5	$Y_5 =14.0 + 0.256 X_1 + 0.166 X_2 + 0.286 X_3 + 8.39 X_4 + 0.43 X_5$ Y_5 = Shamber; X_1 = Max. temperature (°C) X_2 = Min. temperature (°C); X_3 = Relative humidity X_4 = Rainfall (mm); X_5 = Wind speed (Km/h)	98.36
6	$Y_6 = 1.6 + 0.171 X_1 + 0.473 X_2 + 0.041 X_3 + 5.37 X_4 + 0.10 X_5$ Y_6 = Duncan; X_1 = Max. temperature (°C) X_2 = Min. temperature (°C); X_3 = Relative humidity (%) X_4 = Rainfall (mm); X_5 = Wind speed (Km/h)	98.92
7	$Y_7 = 12.7 + 0.610 X_1 + 0.356 X_2 + 0.059 X_3 + 4.51 X_4 + 0.6 X_5$ Y_7 = Foster; X_1 = Max. temperature (°C) X_2 = Min. temperature (°C); X_3 = Relative humidity (%) X_4 = Rainfall (mm); X_5 = Wind speed (Km/h)	97.53
8	$Y_8 = 40.6 + 0.548 X_1 + 0.487 X_2 + 1.042X_3 + 1.85 X_4 + 16.37 X_5$ Y_8 = Malta; X_1 = Max. temperature (°C) X_2 = Min. temperature (°C); X_3 = Relative humidity (%) X_4 = Rainfall (mm); X_5 = Wind speed (Km/h)	98.35
9	$Y_9 = 45.0 + 0.693 X_1 + 0.310 X_2 + 1.154 X_3 + 0.39 X_4 + 14.16 X_5$ Y_9 = Citrus sinensis; X_1 = Max. temperature (°C) X_2 = Min. temperature (°C); X_3 = Relative humidity (%) X_4 = Rainfall (mm); X_5 = Wind speed (Km/h)	98.63
10	$Y_{10} = 24.8 + 0.632 X_1 + 0.699 X_2 + 0.802 X_3 + 3.29 X_4 + 15.20 X_5$ Y_{10} = China lemon; X_1 = Max. temperature (°C) X_2 = Min. temperature (°C); X_3 = Relative humidity (%) X_4 = Rainfall (mm); X_5 = Wind speed (Km/h)	98.08

DISCUSSION

The development of disease depends on the host's susceptibility, the virulence of the pathogen, and favorable environmental conditions. The most important environmental parameters are relative humidity (%), temperature (^oC), rainfall (mm), wind speed (Km/h), and wind speed (Km/s) that manipulated the plants against different diseases

(Bakhsh *et al.*, 2007; Mina and Dubey, 2010). Sudden fluctuations in these environmental conditions can bring drastic effects on plants that lead to develop diseases (Chakraborty and Pangga, 2004). The resistance or susceptibility of plants to pathogens is significantly influenced by these climate factors. They can also alter the host-cause interaction, production, dissemination, infection, and survival of pathogens, as well as the pattern of growth, production, and infection (Saremi *et al.*, 1999; Ghini *et al.*, 2008).

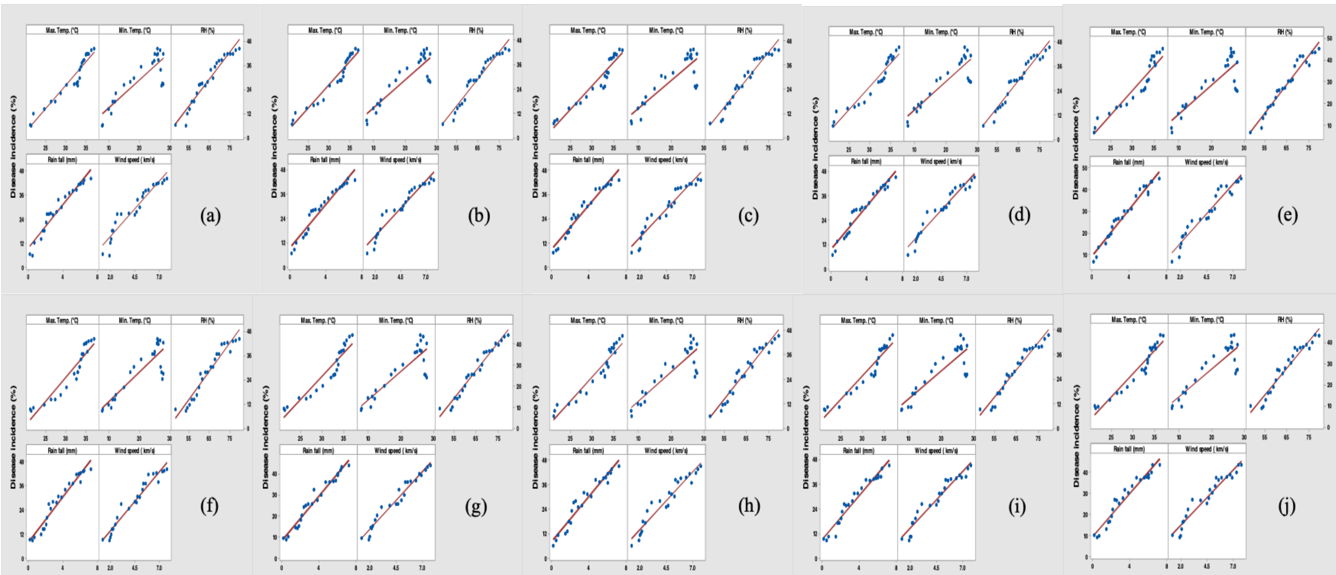

Figure 1. Comparison of environmental factors (max. & min. temp., relative humidity, rain fall, wind speed) and disease incidence (%) through scatter plot on following citrus varieties (a) Grapefruit (b) Rough lemon (c) Kinnow (d) Red blood (e) Shamber (f) Duncan (g) Foster (h) Malta (i) Citrus Sinensis (j) China lemon for 1st year.

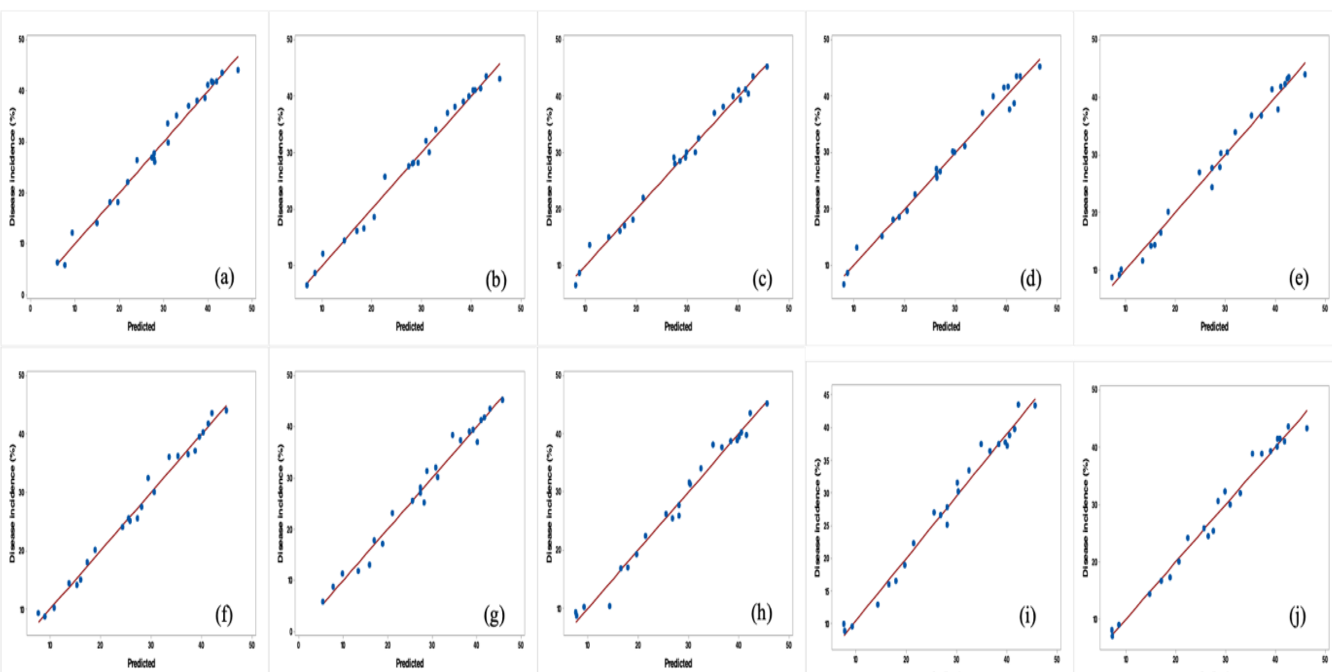

Figure 2. Relation of predicted and observed data values of disease incidence (%) on citrus varieties (a) Grapefruit (b) Rough lemon (c) Kinnow (d) Red blood (e) Shamber (f) Duncan (g) Foster (h) Malta (i) Citrus Sinensis (j) China lemon for 1st year.

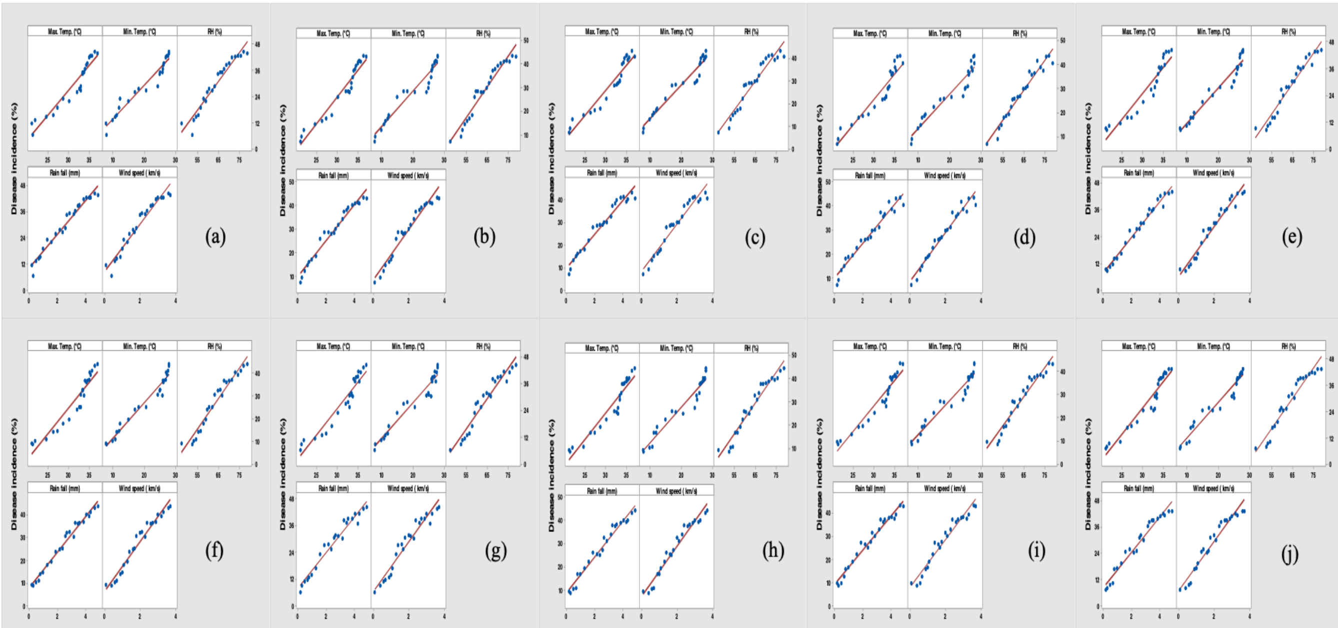

Figure 3. Comparison of environmental factors (Max. & Min. Temp., Relative Humidity, Rain Fall, Wind Speed) and disease incidence (%) through scatter plot on following citrus varieties (a) Grapefruit (b) Rough lemon (c) Kinnow (d) Red blood (e) Shamber (f) Duncan (g) Foster (h) Malta (i) Citrus Sinensis (j) China lemon for 2nd year.

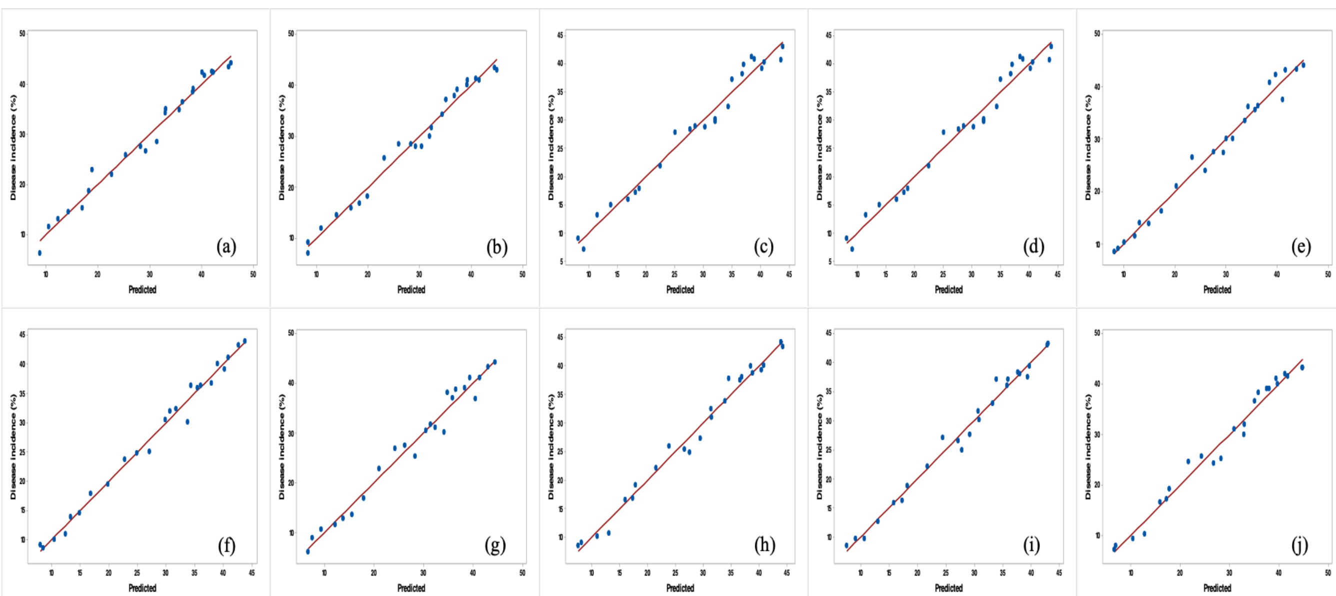

Figure 4. Relation of predicted and observed data values of disease incidence (%) on citrus varieties (a) Grapefruit (b) Rough lemon (c) Kinnow (d) Red blood (e) Shamber (f) Duncan (g) Foster (h) Malta (i) Citrus Sinensis (j) China lemon for 2nd year.

All environmental parameters in the present study showed a strong positive relationship with all of the varieties/cultivars. Highest incidence of disease was developed at 20-28 °C and 30-38 °C (min. and max. T), RH 47-74 % and WS (8 km/h) with 4 mm rain in the first year of study. Similar results were observed for second year. After the pathogen interacted with the plants, environmental factors in the air considerably helped in the disease's development. The development and initiation of plant diseases were significantly influenced by temperature. Numerous pathogens complete their life cycles in the favourable temperature, reproducing quickly (Raaijmakers *et al.*, 2009). Different plant pathogens and diseases have different preferences for colder or warmer temperatures. Others, including *Xanthomonas* and *Ralstonia*, cause canker and bacterial wilts in many plant species and develop significantly more quickly under high temperatures. According to recent studies, high wind speeds can cause spread of pathogen above 50 km/h; by limiting speed of wind in orchards, *Xcc* distribution can be inhibited (Irey *et al.*, 2006; Gottwald and Irey, 2007).

Additionally, there was a strong positive association between rain and the development of disease. With an increase in rainfall, the incidence of canker disease considerably increased (Bock *et al.*, 2005; Khan and Abid, 2007). These results were supported by Gottwald and Irey (2007) who observed that these environmental factors had an important part on the disease development and concluded that maximum temperature with windblown rain was the most important factors. Das *et al.* (2012) also studied the interaction between environmental factors and disease incidence and concluded that percent disease index (PDI) of CC was highest with the increase in temperature (T). Bacterial growth was further promoted by heavy precipitation and strong winds (Christiano *et al.*, 2007). Palazzo *et al.* (1993) studied the prevalence of citrus canker and noticed that disease spreads more rapidly during the summer. He also concluded that long dry period decreases the multiplication of *Xac* on the leaves and fruits. Winds also contribute to the incidence of diseases by pathogens spreading, increasing the lesions and even increasing the drying of moist plant surfaces. Additionally, it helps bacteria to release spores and spread from infected to healthy areas. When active source of inoculums are present, the high winds and rainstorms during the monsoon season also increase the epidemics of numerous bacterial infections (Stall *et al.*, 1993).

CONCLUSION

All citrus varieties have a significant positive correlation with all environmental parameters (Max. T, Min. T, RH, WS, and RF). Continuous environmental monitoring is necessary for accurate citrus canker prediction and control because of sudden changes in the climate. The development of A Decian Support System (DSS) can be used for accurate control of disease based on data gathered from various areas.

COMPETING INTEREST

The authors declare that they have no competing interests.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally to the research and compiling the data as well as editing the manuscript.

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