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OCCURRENCE OF CITRUS TRISTEZA VIRUS IN PAKISTAN: A GIS BASED APPROACH COMBINING HOST DISTRIBUTION AND DISEASE REPORTS

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Citrus tristeza disease is the most devastating viral disease of citrus plants worldwide. It has been emerging as a serious threat to citrus production in Pakistan. Since the first report of CTD in 1988, the disease has been widely distributed in country by now resulting one of the major causes in citrus decline. In this study, the spatial distribution of citrus growing areas, *Citrus tristeza virus* occurrence as well as the effect of environmental factors; temperature and precipitation, has been mapped using geographic information system. Based on the available CTV data from 1992-2013 in Pakistan, subsequent charts were plotted overlaying spatial data of CTV with respect to annual average temperature, record high/low and annual average precipitation. The maximum disease incidence was observed in Sargodha district on an average 67% and minimum of 39% in Mardan. The temperature and precipitation could have been factors driving transmission of CTV locally. The impact of temperature, precipitation and its corresponding CTV incidence in respective year showed a negative relationship of annual average temperature with CTV frequency. However, a positive relationship of precipitation with CTV incidence has been found. The study would contribute to understand the emergence of CTD in this geographical region and to evaluate its extent and extend.
Keywords: Citrus tristeza disease, geographic analysis, information technologies

INTRODUCTION

Citrus fruits are considered the second most important fruits in terms of area and production worldwide. Pakistan is enjoying the 13th position among top 15 citrus producing countries (FAO, 2013). In Pakistan citrus production is spreading on an area of approximately 200,000 hectares producing a yield of over 2 million tons per year (Anonymous, 2011), production mainly concentrated in provinces of Punjab and Khyber Pakhtunkhwa (KP). Mandarin and sweet oranges are the leading cultivars in Punjab and KP provinces, respectively. The citrus fruits are vulnerable to about 30 different viruses and virus like diseases. However, the biggest threat comes from citrus tristeza disease (CTD) caused by *Citrus tristeza virus* (CTV) (Khan, 1992; Iqbal *et al*., 2015; Naqvi *et al*., 2015). CTV belongs to the genus *Closterovirus* and family *Closteroviridae*. It is a non-enveloped particle, bearing filamentous or flexuous virions of diameter 2000 nm and length 11 nm. The genome is positive sense, single stranded RNA of size 20 Kb encapsidated by coat proteins, major and minor respectively (Satyanarayana *et al.,* 2004). The CTV infects all species, varieties and hybrids of citrus, irrespective of the rootstock resulting mild to severe symptoms. It is graft transmissible and not transmitted by seed (Wallace, 1978). The most affected varieties by CTV in Pakistan include Sweet Oranges (Mosambi), Mandarin, grapefruit and lime, respectively (Atta *et al.,* 2011). It has many strains, varied in their genetic and biological diversity ranging from mild isolates to severe stem pitting isolates (Su, 1998; Roberts *et al.,* 2001). CTV is restricted to the phloem tissue of the host and is transmitted naturally in a semi persistent way by means of aphid species that act as a vector (Raccah *et al.,* 1989; Yokomi *et al.,* 1994). The brown citrus aphid (*Toxoptera citridus*) is the most efficient vector in transmitting CTV (Rocha-Peña *et al.,* 1995) but to date, it has not been reported in Pakistan. *Aphis gossypii* and *Aphis spireacola* are the other efficient vector for transmitting CTV in Pakistan (Catara *et al.,* 1988, 1991). Studies on *Aphis gossypii* proved that they could successfully acquire and transmit CTV with a transmission efficiency of 66.6%. The inoculated plants exhibited typical symptoms of chlorosis and vein clearing after a period of 4-6 weeks (Arif *et al.,* 2005). The aphid's survival mainly depends on their local climate because they have restricted ability to regulate body temperature (Bale and Hayward, 2010). It is known that warmer winters have a positive effect on the annual number of aphids (Hazell *et al.,* 2010). Studies have also shown that aphids are intolerant to higher temperature. The temperature ranging from 41-42°C can lead to heat coma and death of an aphid. However, aphid survival depends on precipitation (ppt.) too (Kleuken *et al.,* 2009). The precipitation in the form of heavy rain can wash aphids from the air (Lewis and Siddorn, 1972), considered as most effective to clean the air. Moreover, heavy rains immediately after aphid fallout may even kill newly arrived aphids on the plant (Parry, 2013). A study conducted by Mann

et al., (1995), concluded that ppt. in the form of heavy rain dislodged large number of aphids and they traveled long distance as compared to those exposed to drizzle or light rain. Contrarily, Zhang (2002) observed the presence of apterous aphid on plants throughout the precipitation period and once the precipitation even ceased, similar number of apterae (wingless aphid) left plants irrespective of the intensity or duration of the precipitation event and yet traveled the same distances. The mechanism of impact of humidity is unclear, whether directly on flight behavior of aphids or indirectly on its reproduction (Kleuken *et al.,* 2009).

The major citrus growing areas in Pakistan have shown heavy CTV infections in recent years indicating CTV as an emerging threat to citrus industry in the country. The CTV is widespread particularly in new plantations due to uncertified and infected bud wood as a consequence of which even new plantation would be unsuccessful until a pathogen free bud wood is supplied to citrus growers. As a result of this, the citrus industry in Pakistan is potentially losing billion's worth in foreign exchange. Furthermore, with no rules of production and international certifications the problem remains dominant. Despite recognition on the importance of certification, the Agri Business Support Global Project (GAP), launched in 2006 in an attempt to bring farmers under international certification of Global GAP sadly lost its momentum in subsequent years. GIS (Geography Information System) is an extremely powerful tool in bringing agricultural management to new heights. It has been used for the spatial analysis of insect pest and disease (Nelson *et al.,* 1994). Especially, the use of GIS technology in agriculture management is termed as "Geophytopathology" (Weltzien, 1988). It allows us to establish spatial patterns for plant diseases; the understanding of those patterns as well as the various geographic aspects that impact disease control. GIS maps allow detailed description and analysis of the spread of plant diseases, which can be on a local, regional or continental scale.

The primary objective of this study was to illustrate the spatial distribution of CTV disease in conjunction with temperature and ppt. associated with the spread of disease in major citrus growing regions of Pakistan.

MATERIAL AND METHODS

***Citrus tristeza virus data*:** The status of Citrus tristeza virus reported in Pakistan has been established by collecting available data from 1992-2013 for the Punjab and Khyber Pakhtunkhwa provinces (supplementary Tables 1 and 2). The existing studies on CTV occurrence include samples from commercial citrus orchards and nurseries. The incidence of CTV, in newly planted trees has not been specified, and sampling in some studies was done on the basis of surveys (Catara *et al.,* 1988 and 1991; Anwar and Mirza, 1992), diagnostic symptoms, random sampling (Arif *et al.,* 2005), or orchards that met specific criteria such as a traditional farming system, minimum 200 number of living citrus trees, orchards aged 15-20 years (Iftikhar *et al.,* 2009). Whereas, one study (Atta *et al.,* 2011), considered only orchards meeting criteria of 10 years or more. The methods used for detection of CTV also varied, utilizing Electron microscopy (Catara *et al.,* 1988, 1991; Arif *et al.,* 2005), ELISA (Arif *et al.,* 2005; Iftikhar *et al.,* 2009) and Direct Tissue Blot Immuno Assay (DTBIA) (Atta *et al.,* 2011).

Table 1. Correlation between temperature, precipitation and CTV incidence in Punjab province-Pakistan.

Variable	Ann. Av. Temp (°C)	Ann. Av. Max. Temp (°C)	Ann. Av. Min Temp (°C)	Total ppt (mm)	% CTV Incidence
Ann. Av. Temp (°C)	1.00	0.77	0.69	0.17	-0.12
Ann. Av. Max. Temp (°C)	0.77	1.00	0.17	-0.03	-0.17
Ann. Av. Min. Temp (°C)	0.69	0.17	1.00	0.35	0.01
Total ppt (mm)	0.17	-0.03	0.35	1.00	0.74
% CTV Incidence	-0.12	-0.17	0.01	0.74	1.00

Ann. – Annual, Av. – Average, ppt - Precipitation

Table 2. Correlation between temperatures, precipitation and CTV incidence in Khyber Pakhtunkhwa (KP) province-Pakistan.

Variable	Ann. Av. Temp (°C)	Ann. Av. Max. Temp (°C)	Ann. Av. Min Temp (°C)	Total ppt (mm)	% CTV Incidence
Ann. Av. Temp (°C)	1.00	0.99	0.98	-0.96	-0.24
Ann. Av. Max. Temp (°C)	0.99	1.00	0.96	-0.96	-0.17
Ann. Av. Min. Temp (°C)	0.98	0.96	1.00	-0.94	-0.32
Total ppt (mm)	-0.96	-0.96	-0.94	1.00	0.31
% CTV Incidence	-0.24	-0.17	-0.32	0.31	1.00

Ann. – Annual, Av. – Average, ppt. - Precipitation

Environmental data: For data collection of environmental variables including temperature and precipitation (ppt), the locations were selected for reported CTV incidences in both Punjab and KP provinces. The most consistent year wise climatic data was acquired for temperature and ppt values from online sources including Google Earth; Berkeley Earth; Tutiempo; Climate-Data; Pakmet and Weather base (all accessed in April 2014).

Spatial and statistical modeling: In GIS software, QGIS (Quantum Geography Information System) the district level shape file of only four provinces of Pakistan (Punjab, Khyber Pakhtunkhwa, Sindh, Balochistan provinces) has been imported. To map the citrus growing fields, data has been used from AMIS (Agricultural Marketing Information System) presenting tabular district wise data for citrus area and production (2007-09) with the average value being used. The major citrus growing regions in Pakistan have been mapped based on citrus production (> 720 tons) in different districts.

Using QGIS Join feature the attribute (tabular) data has been linked with spatial data showcasing the various ranges of citrus producing regions in Pakistan. These regions are then represented spatially by allocating random points within identified locations by utilizing research tools under vector. The spatial data for distribution of CTV was imported from supplementary Table 1 and 2 in QGIS attribute table of Pakistan administrative level 2 districts shape file. Mean Temperature, record high temperature, low temperature and annual precipitation maps were created overlaying the spatial distribution of CTV infected regions to investigate role of temperature and precipitation on CTV distribution. For statistical analysis, year wise data of annual average temperature, annual average maximum (max.) temperature, annual average minimum (min.) temperature and precipitation were gathered and compiled in Microsoft Excel sheet as CTV incidence in the particular reported region along with percentage in the corresponding year. To investigate relationship of these environmental variables with CTV incidence (%) correlation matrices were generated using STATISTICA software version 8.0 [StatSoft Inc., USA).

RESULTS

Main citrus fruit production districts in Pakistan have been illustrated in Figure 1. The major districts with citrus production in Punjab province are; Sargodha, Mandi Bahauddin, Toba Tek Singh (TTS), Sahiwal, Khanewal, Vehari, Bahawalpur and Multan. Whereas in KP province, the districts, contributing significantly are; Malakand, Swat, Nowshera, Lower Dir, Dera Ismail Khan (DIK) and Mardan.

The maps showing average % CTV incidence in Punjab (Fig. 2) and in KP (Fig. 3) on a district and tehsil level were created using available data compiled in supplementary Table 1 and 2. The highest incidence (67%) was recorded in Sargodha district. An even higher prevalence of CTV was recorded in Tehsils Bhalwal and Kot Momin of Sargodha district with maximum CTV incidence of 53% and 100%, respectively. The mean CTV disease incidence of Bhalwal and Kot Momin was the highest in the province being 37.64% and 67%, respectively (Fig. 2).

The highest CTV incidence in KP was observed in Mardan 40.86% followed by Haripur and Swabi with an incidence rate of 40%. The highest mean incidences of CTV was in Swabi and Mardan with 37.5% and 31.55%, respectively (Fig. 3).

Once the citrus producing areas and the CTV occurrence areas were identified, the data was represented spatially (Fig. 4).

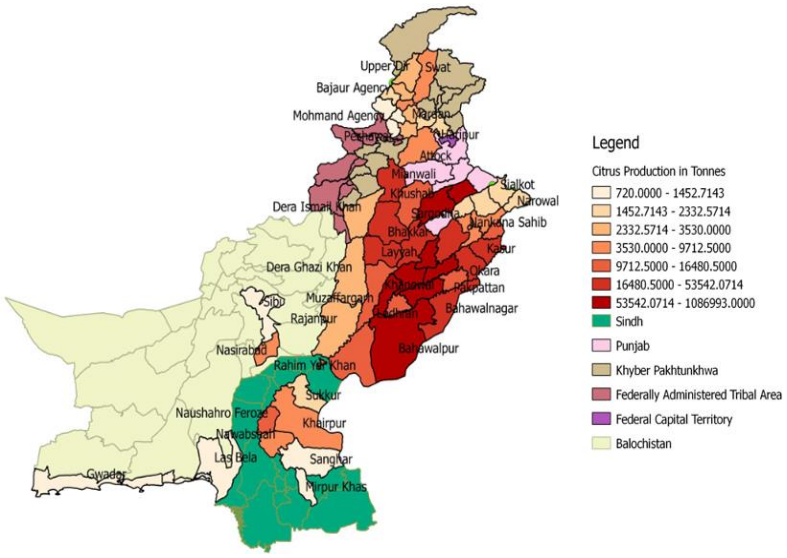

Figure 1. Major Citrus Producing Districts in Punjab, Khyber Pakhtunkhwa (KP), Balochistan and Sindh provinces of Pakistan.

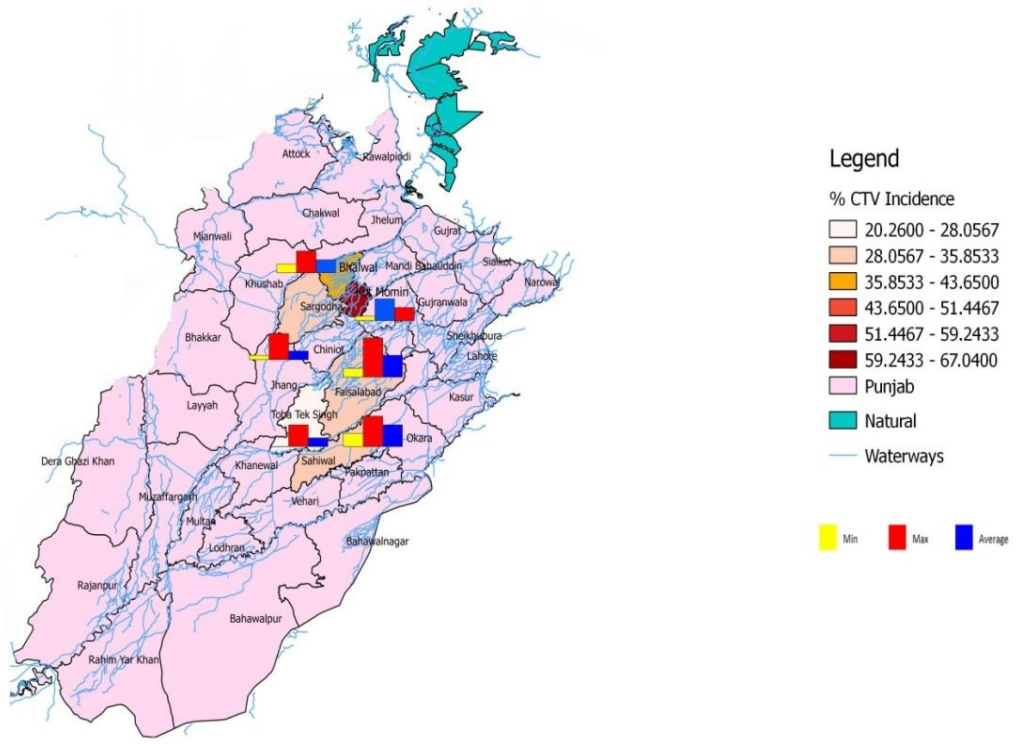

Figure 2. Average % CTV incidence in the Punjab Province-Pakistan. The yellow, red and blue boxes show the min. max. and average % CTV incidence, respectively in different cities.

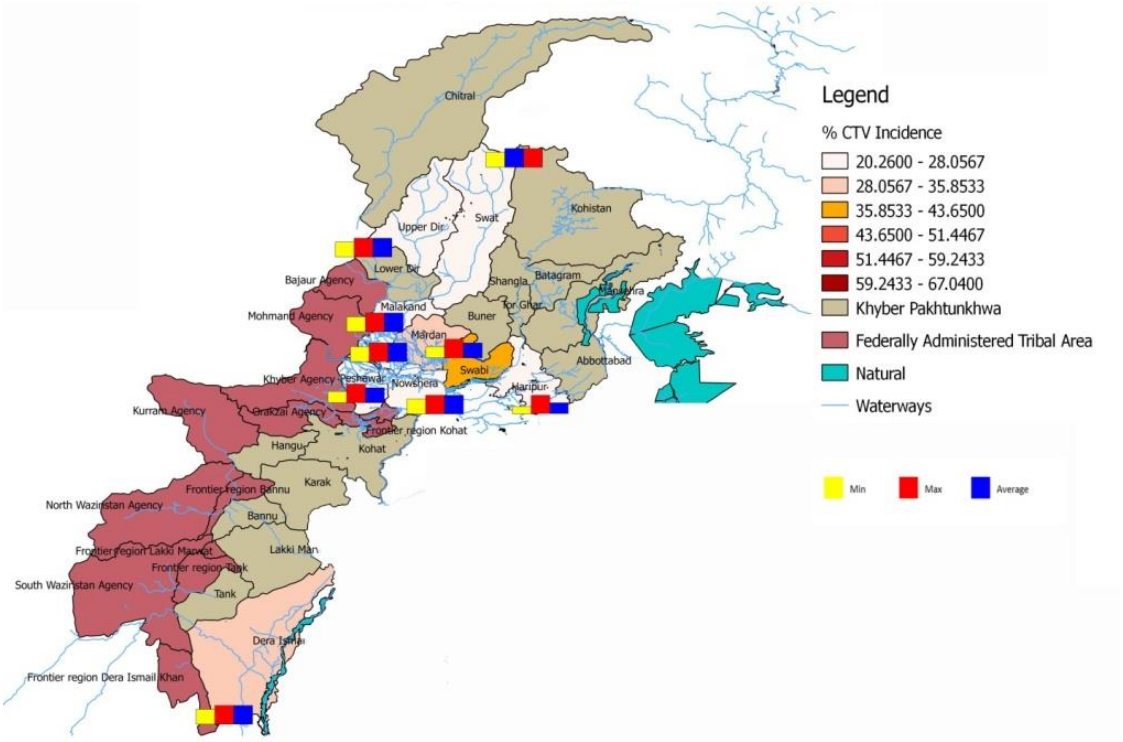

Figure 3. Average % CTV incidence in the KP Province-Pakistan. The yellow, red and blue boxes show the min. max. and average % CTV incidence, respectively in different cities.

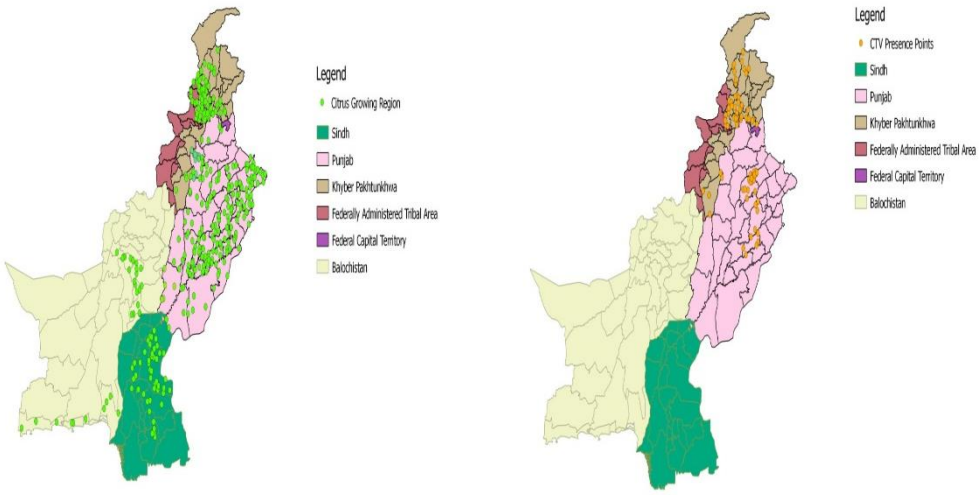

Figure 4. Spatial distribution of citrus growing areas and *Citrus tristeza virus* (CTV) occurrence in Punjab, KP, Sindh and Balochistan Provinces in Pakistan. a) Citrus growing area (marked as green dots), b) CTV occurrence (marked orange dots)

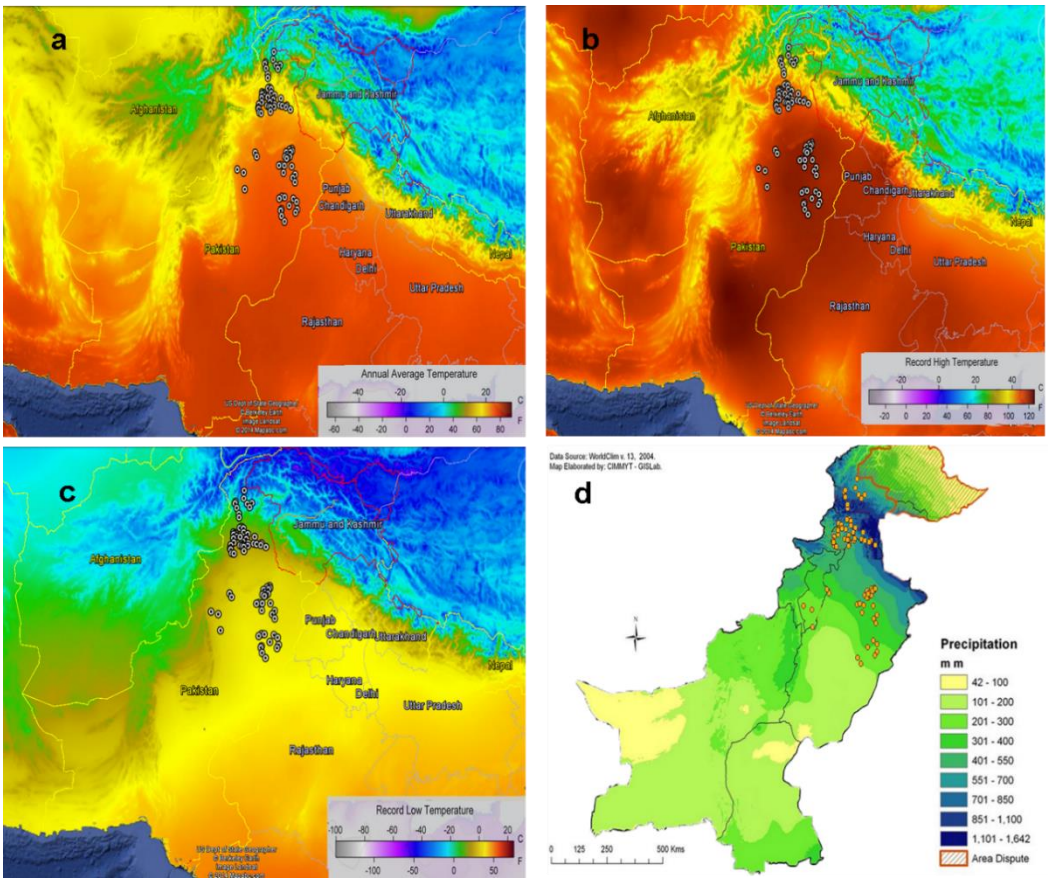

Figure 5. Spatial distribution of *Citrus tristeza virus* (CTV) infection in relation to temperature and precipitation in four provinces of Pakistan. a) Spatial distribution of CTV infection with annual average temperature, b) Spatial distribution of CTV infection with record high temperature, c) Spatial distribution of CTV infection with record low temperature, d) Spatial distribution of CTV infection in relation to precipitation.

Table 3. % CTV incidence in Punjab Province-Pakistan (Supplementary).

Regions in Punjab (city)	1992 (Anwar and Mirza,1992)	2005-06 (Abbas *et al*., 2008)	2006 (Iftikhar *et al*., 2009)	2007 (Iftikhar *et al*., 2009)	2008 (Atta *et al*., 2008)	2011-12 (Atta *et al*., 2011)	2012-13 (Jalil, 2013)	Min. %	Max. %	District wise Ave. %
Faisalabad	13.13	12.95	21.53	23.84	35.50	35.50	----	12.95	50.00	28.45
Sahiwal	18.18	02.90	28.46	30.00	36.22	18.11	----	18.11	44.44	29.13
T.T. Singh			16.15	16.92	23.61	17.71	----	00.00	37.50	20.75
Sarghoda	13.20	32.30	22.30	24.61	28.60	28.59	67.00	13.20	67.00	30.09
Bhalwal			44.61	48.46	34.08			25.00	53.00	37.64
Kot Momin	----	----	----	----	----	34.08	100.0	34.10	100.0	67.04
Islamabad					----	----	0.00	----	----	00.00
Chak 9 Shumali	----	-----	----	----	-----	-----	67.00	----	----	67.00
Year wise Avg.	15.04	24.75	26.61	28.77	31.60	26.80	58.50	15.05	58.50	

---- data not available

Table 4. % CTV incidence in Khyber Pakhtunkhwa (KP) province-Pakistan (Supplementary).

Regions in KP (city)	2001-2003 (Arif *et al*., 2005)		2006 (Iftikhar *et al*., 2009)	2007 (Iftikhar*et al*., 2009)	Min.	Max.	District Wise (Average %)
Peshawar	23	27	23.47	29.56	23	29.56	25.76
Nowshera	25	28	----	----	25	28.00	26.50
Charsadda	28	22			22	28.00	25.00
Mardan	28	25	37.39	40.86	25	40.86	32.81
Swabi	35	40	----	----	35	40.00	37.50
Haripur	18	18	31.30	40.00	18	40.00	26.82
Malakand	29	23	---	----	23	29.00	26.00
Swat	26	30	----	----	26	30.00	28.00
Dir	27	23	----	----	23	27.00	25.00
D.I. Khan	33	26	----	----	26	33.00	29.50
Year Wise Avg.	26.7		30.72	36.80667			

---- data not available

Table 5. Relationship between temperature, precipitation and % CTV incidence in regions of Punjab province-Pakistan (Supplementary).

City	Year	Ann. Av. Temp. (°C)	Ann. Av. Max. Temp (°C)	Ann. Av. Min. Temp (°C)	Total ppt (mm)	% CTV Incidence
Faisalabad	1992	24.29*	31.00*	17.58*	401.90*	13.13
	2005	23.98	30.51	18.18	223.29	12.95
	2006	24.83	31.29	18.84	432.82	21.53
	2007	24.89	31.94	17.79	344.68	23.84
	2008	24.02	30.92	17.32	525.50	35.50
	2011	24.00	30.60	17.6	488.17	35.50
	2012	24.00	30.90	17.2	371.09	35.50
Sargodha	1992	23.81	29.88	17.51	57.92	13.20
	2005	24.19	30.33	18.21	328.46	32.30
	2006	25.16	31.23	18.99	525.01	22.30
	2007	24.57	31.16	17.89	679.97	24.61
	2008	24.86*	31.20*	18.44*	602.49*	28.60
	2011	24.35	30.84	18.26	730.24	28.59
	2012	24.27	30.86	17.70	366.58	47.80*
	2013	24.27	30.33	18.48	1574.81	67.00

*Average Values Considered; Ann. – Annual, Av. – Average, ppt - Precipitation

Table 6. Relationship between temperature, precipitation and % CTV incidence in regions of Khyber Pakhtunkhwa (KP) province-Pakistan (Supplementary).

City	Year	Ann. Av. Temp (°C)	Ann. Av. Max. Temp (°C)	Ann. Av. Min. Temp (°C)	Total ppt (mm)	% CTV Incidence
Peshawar	2001	23.11	29.79	17.48	165.85	23.00*
	2002	23.06	29.48	17.98	446.02	27.00*
	2003	23.08*	29.63*	17.73*	305.93*	25.00*
	2006	23.23	29.20	18.04	453.14	23.47
	2007	22.90	29.50	16.80	590.28	29.56
D. I. Khan	2001	24.69	31.68	18.60	250.68	33.00*
	2002	25.51	32.62	18.50	87.89	26.00*
	2003	24.63	31.48	17.52	267.48	29.50*
Dir	2001	15.77*	24.09*	8.27*	1155.97*	33.00*
	2002	15.49*	23.32*	8.56*	1306.86*	26.00*
	2003	15.66*	23.20*	7.80*	1033.77*	29.50*

*Average Values Considered. Ann. – Annual, Ave. – Average, ppt - Precipitation

The spatial distribution of CTV presence was then used to create further maps overlaying average annual temperature, record high and low temperature and average annual precipitation amount (Fig. 5 a-d).

The incidence of CTV in each year (based on available data only) and its respective temperature and precipitation data for regions in Punjab and KP provinces is compiled in supplementary tables 3 and 4. The correlation matrix generated (Table 1 and 2) on the basis of data presented in supplementary tables 3 and 4 showed a negative correlation between average annual, maximum and minimum temperatures in districts of KP. While, in the regions of Punjab, the annual and maximum temperatures are negatively correlated with minimum temperature showing a positive correlation with the disease incidence.

DISCUSSION

Citrus tristeza virus (CTV) is causal agent of an economically important and destructive tristeza disease of citrus in Pakistan. Major portion of citrus production in the country comes from the sub-tropical continental low land type (including Punjab province), which generally has semi-arid and extreme climate. However, a small portion of citrus production resides in sub-tropical high land type (including KP province) and a relatively minor production is from rest of the regions in the country. The CTV is transmitted by insect vector and propagation of infected bud wood. The use of surveillance methods and technologies including GIS is now pivotal for plant disease study and management thus for plant biosecurity (Kalaris *et al*., 2014). The mapping of CTV occurrence in major citrus growing areas of Pakistan by using a GIS based approach combining disease reports and host distribution is a promising approach. A correlation of environmental factors including temperature and precipitation on disease occurrence showed that the CTV is wide spread in Punjab province than KP. The low incidence in KP could be due to cold temperature particularly in northern regions of the province. The maximum disease incidence was observed in Sargodha district on an average 67%. No disease incidence has been reported in citrus growing orchards of Islamabad (Federal capital). The CTV prevalence was found high particularly in Mardan and Swabi districts of KP, having lesser rainfall and higher temperatures as compared to northern part of KP. The wide spread occurrence of CTV could be correlated with warmer annual average temperature about 21.5-25°C (Berkley Earth, 2014) and high average rainfall ranging from 300-1200 mm which was more suitable environment for aphid survival and transmission. However, considering the temperature trends of Pakistan, it was quite likely that anthropogenic activities of the human beings were responsible for the spread of CTV further and faster than aphids. The negative correlation of high temperature with % CTV incidence suggested that in summer seasons, heat hindered aphid ability to survive particularly in the province of Punjab, which experienced higher temperatures in comparison to KP province. Aphid reproduction and survival would be significantly reduced if temperature remains high for longer periods i.e. above optimal threshold for aphid growth (Qureshi, 2010). On the other hand, in winter season, the KP province has temperature range close to subzero range, which made it unfavorable for aphids to survive while the higher temperatures in Punjab favored aphid and other insects survival (Shah *et al*., 2015).

There have been inconsistent studies investigating the relationship of ppt. and its effect on aphid dispersal as well as in spread of disease. The analyses showed a positive correlation between precipitation in regions of KP as well as Punjab (Tables 1 & 2). Thus, it can be assumed that when the annual and maximum temperature increased, the CTV incidence was low probably because it hindered aphid survival. Similarly, when the minimum temperature was very low it also obstructed aphid survival and consequently CTV transmission by aphids (Qureshi, 2010).

The positive correlation of precipitation and CTV disease in this study was consistent with earlier studies, which suggested that CTV incidence was positively impacted by precipitation due to greater dislodge and distances travelled by aphids, hence played a role in its transmission (Mann *et al.,* 1995). The ideal temperature for aphids is about 22°C and generally no growth and development of aphids would occur below 5°C or above 33°C.The temperatures in Northern KP were known to reach subzero levels particularly in the winters; however, Southern KP and Punjab had warmer temperatures. The warmer winter was likely to improve aphid survival. However, extreme summer temperature reduced reproduction and nymphal survival. In Pakistan, the summer temperature exceeds above 30°C with mean temperature during peak month of June (38°C). The temperature beyond 28°C would negatively impact aphid reproduction and nymphal survival. The temperature as high as 50°C had been reported in Southern KP as well as in Punjab, as recorded in regions of DI Khan, Peshawar, Bahawalnagar and Sargodha (Pak Met). Despite such high ambient temperatures small colonies of aphids were able to survive due to protected microclimate as reported by Tsai and Wang (1999).

Most of the regions in Pakistan are showing positive trend in temperature rise for the period 2011-2050. The maximum rise is expected in Northern areas of the country and Central Southern Punjab as well as Lower KP (Chaudhary *et al.,* 2009). It can therefore be inferred that any significant increase in winter temperatures minima would positively impact aphid survival thus possibly leading to more widespread outbreak in winters. In Pakistan, CTV poses a threat not only economically but also ecologically, hence a serious threat to the biodiversity of an agricultural country like Pakistan. Hence, it is recommended to take urgent and serious initiatives for CTV surveillance and epidemiology to control the disease. The progressing trend in CTV incidence is likely to be attributed to usage of infected bud wood and existence of various old orchards, which act as virus reservoirs. Based on the study, a future recommendation would be to slowly shift citrus production areas i.e. non-traditional areas as well.

***Conclusion*:** The study will contribute to understand the dynamics of citrus grown in Pakistan and its relationship with the environmental conditions to determine the extent to which *Citrus tristeza virus* may persist or disappear. To reflect the actual exposure of host plant to any pathogen, a combination of predictive distribution maps of the host and disease cases, represents a step forward in the understanding of the disease. Therefore, we propose thematic maps at national scale, for surveillance of plant diseases to forecast the disease and to implement an early diagnosis and management plan.

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