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PREVALENCE, OCCURRENCE AND DISTRIBUTION OF CHILI VEINAL MOTTLE VIRUS IN PAKISTAN

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Abstract

Chili veinal mottle virus (ChiVMV) is one of the prevalent chili-infecting virus found throughout chili growing areas of Pakistan. Disease monitoring during 2003 and 2004 showed 44.7% relative occurrence of the virus in the country. In the year 2003, the relative occurrence was 48, 51, 41 and 38% in Sindh, Punjab, NWFP and Balochistan whereas during 2004, the relative occurrence was 47, 44, 41 and 34% respectively in the provinces. Highest relative occurrence was recorded in Sindh (47.3%) and Punjab (46.9%) followed by NWFP (41.3%) and Balochistan (35.7%). No district was found free from the viral infection in Sindh and Punjab except from few locations of NWFP (Dir, Bajour Agency, and Lakky Marwat District) and Balochistan (Kachi and Loralai districts). Among weed flora of chili pepper, *Datura metel* was found in Punjab province. Moreover, among insect vectors, *Aphis gossypii* were observed in NWFP during surveys.

Introduction

Capsicum (*Capsicum annum* & *C. frutescens*) is among the world's most popular vegetable belonging to family *Solanaceae* after potato and tomato and is being used mainly as spices and condiments (Berke, 2002). In Pakistan, chili is a significant cash crop among vegetables. The crop occupies 19% of the total area under vegetable cultivation and its production area is mainly concentrated in Sindh (46917 ha) and Punjab (6400 ha), and on small scale in Balochistan (2082 ha) and NWFP (392 ha) (Fig. 1). As the climatic conditions of Pakistan are diverse, chilies are grown at different times in different ecological zones in each province. During 2004-05, the total cropped area under chili cultivation was 48700 hectares, producing 90500 tons with an average national yield of 1.9 tons per hectare. Sindh produces 85% of the chili crop followed by Punjab with 11% (Anon., 2004-05).

Viruses have been found as the most devastating disease causing agents of chili pepper, causing serious losses (Kang *et al.*, 1973; Lockhart & Fischer, 1974; Villalon, 1975; Ong *et al*., 1980; Yoon *et al.*, 1989; Chew & Ong, 1990). Chili pepper is naturally susceptible to a wide range of viruses in all South Asian countries including Pakistan, (Green, 1991; Hameed *et al*., 1995; AVRDC, 2001). So far 65 viruses have been reported infecting chili crop throughout the world (Green & Kim, 1994; Anon., 2001). In Pakistan, initially a number of viruses were detected and identified of which ChiVMV and Cucumber mosaic *virus* (CMV) were identified in 1995 on chili pepper during surveys of different provinces with highest incidence (Hameed *et al*., 1995). The present study was conducted to monitor disease pattern of the virus throughout the country, to assess the crop condition as well as to collect information regarding prevalence, distribution and occurrence of ChiVMV so that management strategies can be devised to minimize crop losses.

Material and Methods

Surveys and sample collection: Surveys of farmer's fields were conducted during crop season in major chili growing areas of four provinces of Pakistan i.e., Punjab (Attock, Rawalpindi, Jehlum, Chakwal, Talagang, Gujranwala, Lahore, Okara, Sargodah, Mianwali, Sahiwal, Faisalabad, Jhang, Cheniot, Vehari, Sailkot, Narowal, Bhurewala, Khanewal, Multan, Bahawapur, D.G. Khan, Muzafargarh, Rahim Yar Khan and Lodhran), Sindh (Karachi, Thatta, Hyderabad, Larkana, Dadu, Shikarpur, Ghotki, Sanghar, Sukkur, Khairpur, Nawabshah, Mirpurkhas, Jaocobabad and Halla), NWFP (Malakand Agency, Swat, Charsadda, Mardan, Peshawar, Shinkyari, Haripur, Mansehra, Kohat, Karak, Bannu, Lakki Marwat, Hangue and D.I. Khan) and Balochistan (Quetta, Sibbi, Kalat, Mastung, Kachi and Loralai) during 2003-04. Sampling was done in such a way that *Capsicum* (red & sweet) leaves showing mottling, mosaic, deformation, necrosis and narrowing were collected by moving across the field diagonally. The cut portion of branch bearing leaves was wrapped in distilled water-wetted cotton, placed in polythene bags and stored in ice-box.

Virus detection: The collected samples were tested through Double Antibody Sandwich Enzyme Linked Immunosorbent Assay (DAS-ELISA) for ChiVMV, CMV, Tobacco mosaic virus (TMV) and Potato virus Y (PVY) as described by Clark & Adams (1977). The relative occurrence was determined by applying proportionate test (Steel & Torrie, 1980). Similarly weeds flora growing in chili fields were also collected and tested for ChiVMV presence after identifying taxonomically. Later ChiVMV-infected samples were inoculated onto indicator plants of tobacco (cv. Samsun) to maintain and propagate the collected isolates *In vivo* under glasshouse conditions. Moreover, for the isolation of pure ChiVMV isolates from survey-collected samples, method of Green (1997) was adopted.

Results

Monitoring of ChiVMV: Main chili growing areas of the country are shown in Fig. 1. Chili pepper growing season varies throughout the different ecological zones in the country. The crop is mostly cultivated in summer season throughout the country but in Sindh province the crop is grown in autumn/spring season also. Most of the surveys were conducted at flowering/fruiting stage. Aphids were observed during sample collection, feeding on the underside of the leaves mostly early in the morning that was identified as *Aphis gossypii* at Insect Pest Management Program, NARC, Islamabad.

In 2003 growing season, relative occurrence of ChiVMV was 48, 51, 41 and 38 % in Sindh, Punjab, NWFP and Balochistan, respectively; whereas during 2004, relative occurrence was 47, 44, 41 and 34 % in Sindh, Punjab, NWFP and Balochistan respectively. The ChiVMV incidence was higher (>50 %) in Punjab but decreased in the following year 2004. Similar was the situation in Balochistan but in the other two provinces (Sindh and NWFP) the relative occurrence remained the same. Thus average relative occurrence of ChiVMV determined in each province was 44.7 %. The cumulative data of two years showed that highest relative occurrence of ChiVMV was recorded in Sindh (47.3%) and Punjab (46.9%) followed by NWFP (41.4%) and Balochistan 35.7% (Fig. 2). The overall picture showed that ChiVMV was distributed whereever *Capsicum* is grown with few exceptions in some locations in NWFP and Balochistan where no virus was detected in any sample tested.

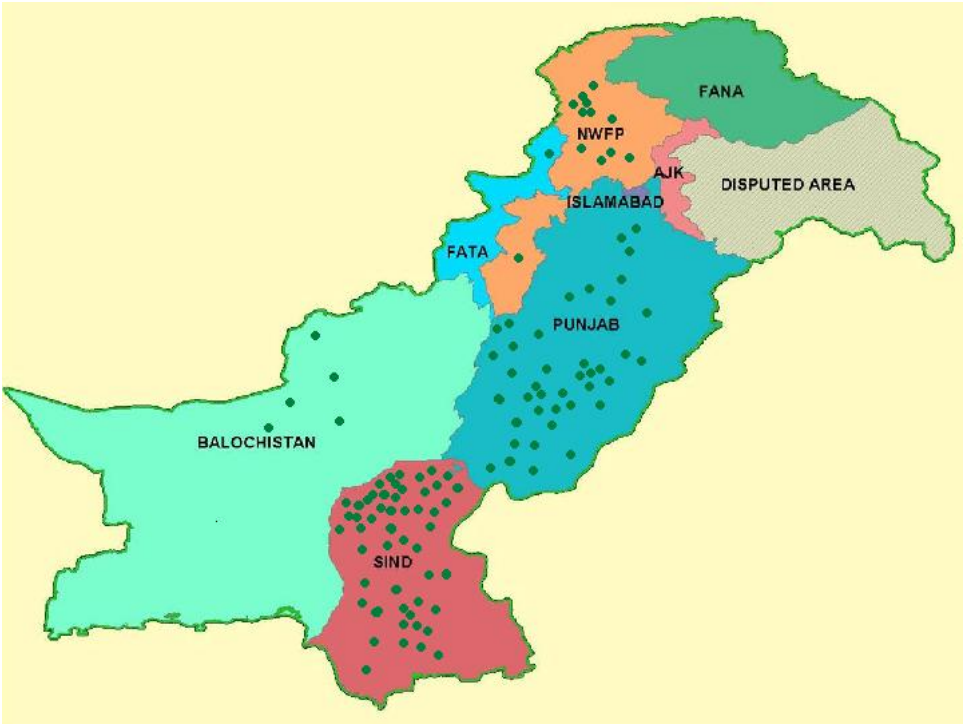

Fig. 1. Map showing chili pepper growing areas of Pakistan.

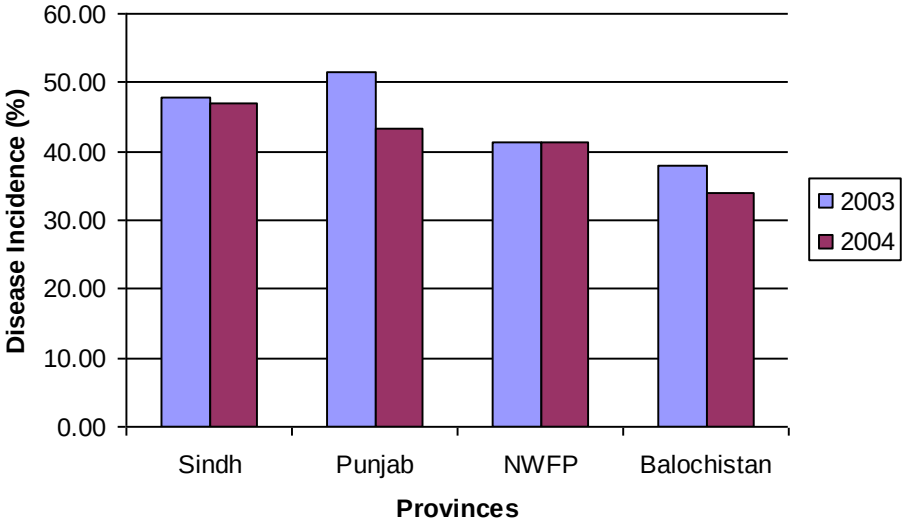

Fig. 2. Province wise relative occurrence of ChiVMV during 2003 and 2004.

The area under bell pepper was higher than hot pepper in the province of Punjab but in Sindh province the cultivation of hot pepper was dominant. Sindh is popular for spices, condiments and pickle as 85 % of chili pepper crop is grown in this province only and Asia's biggest chili market is situated in Kunri district of the province. In case of NWFP, both hot and bell types were cultivated in northern parts of the province particularly in Malakand Agency and district Swat but mostly dominated by bell pepper. However, in southern parts of the province (Karak, Kohat, Lakky Marwat, Bannu & D.I. Khan), hot chili types were grown on more acreage and that is why quality of hot spices and condiments of Bannu and D.I. Khan are famous throughout the province. In case of Balochistan province, the hot pepper was cultivated on more area than sweet pepper.

Symptoms observation under natural field conditions: The symptoms observed during field surveys included mild to severe vein mottling, mosaic, leaf narrowing, malformation and stunted growth of chili plants (Fig. 3). The disease syndrome was uniform everywhere in all the *Capsicum* growing areas of the country. On young seedlings, vein mottling were observed but lateral leaves of infected plant were narrower than older ones. Similar observations were obtained in glasshouse conditions on both *Capsicum* types when inoculated. Both types were found equally susceptible during these surveys but majority of samples from bell pepper were found infected in Malakand Agency and district Swat of NWFP and almost all districts of Punjab province.

Prevalence, distribution and relative occurrence of ChiVMV: *Sindh*. Hot chili pepper is mostly cultivated in Sindh province throughout the year due to its diverse agro-ecological conditions. The survey data of 2003 revealed that relative occurrence of ChiVMV was 42.3, 33.3, 42.5, 42.9, 65.4, 55.9, 62.1, 31, 29.6, 70.4, 33.3, 59.1, 60.7 and 14.4 % in Karachi, Thatta, Hyderabad, Larkana, Dadu, Shikarpur, Ghotki, Sanghar, Sukkur, Khairpur, Nawabshah, Mirpurkhas, Jaocobabad, and Halla respectively and in the following year 2004, the relative occurrence of ChiVMV was 42.1, 25.5, 45.6, 40.5, 70.1, 60.5, 58.4, 28.2, 36.6, 65.7, 27.9, 65.8, 55.9 and 52.6 % in Karachi, Thatta, Hyderabad, Larkana, Dadu, Shikarpur, Ghotki, Sanghar, Sukkur, Khairpur, Nawabshah, Mirpurkhas, Jaocobabad and Halla respectively. Overall relative occurrence was 47.3 and 46 % in 2003 and 2004, respectively (Fig. 2). Highest relative occurrence of ChiVMV was recorded in Khairpur district (70.4%) followed by Dadu (65.4%), Ghotki (62.1%) and Jacobabad (60.7%) while the rest of districts had relative occurrence less than 60% i.e. Mirpurkhas (59.1%), Shikarpur (55.6%) during 2003 (Fig. 4). In the year 2003, highest relative occurrence of ChiVMV was recorded in Khairpur, Dadu, Ghotki, Jacobabad, Mirpurkhas and Shikarpur but in 2004 highest relative occurrence was recorded in Dadu, Mirpurkhas, Khairpur and Shikarpur districts. None of the surveyed areas in Sindh province was found free from ChiVMV infection during these two consecutive years of surveys.

It seems that ChiVMV persists because foci of inoculum remain there and shift of inoculum from one area to other might perpetuate this virus unless proper integrated disease management is adopted. Moreover, long distance dispersal of viruliferous aphid by wind or transportation of infested materials by human activities is likely to spread this or other viruses to new cultivating areas. The collected samples of weeds flora from chili pepper crop in the province included *Cynodan dactylon*, *Cyprus rotundus*, *Desmostachya bipinnata*, *Salvadora oleoides*, *Echinochloa* sp., and *Withania somnifera* and when tested through DAS-ELISA were not found infected with ChiVMV.

Fig. 3. Chili pepper plant showing characteristic symptoms of ChiVMV.

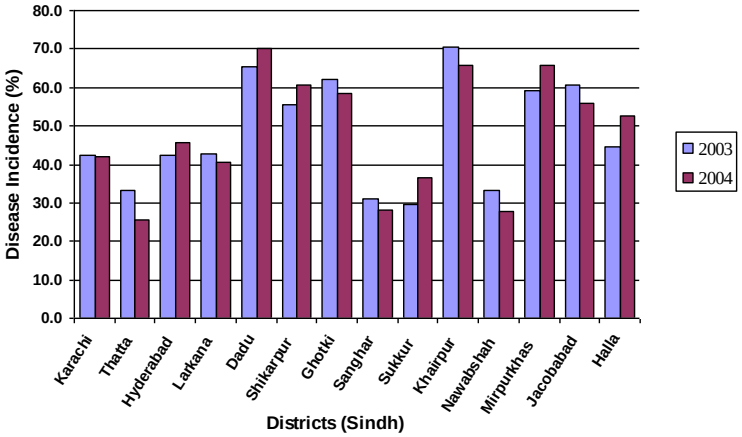

Fig. 4. Prevalence, distribution and relative occurrence of ChiVMV in major chili growing districts of Sindh during 2003 & 2004.

Punjab: In Punjab province bell pepper (*Capsicum annum*) is mostly cultivated in all districts. The data revealed that during 2003, incidence of ChiVMV recorded was 31, 42.3, 64.3, 55.6, 43.8, 52, 41.7, 36.4, 46.2, 35.7, 46.2, 41.7, 36.4, 63.6, 40, 25, 36.4, 41.7, 28.6, 64.7, 56.3, 62.1, 69.2, 47.1 and 44.4% in Attock, Rawalpindi, Jehlum, Chakwal, Talagang, Gujranwala, Lahore, Okara, Sargodah, Mianwali, Sahiwal, Faisalabad, Jhang, Cheniot, Vehari, Sailkot, Narowal, Bhurewala, Khanewal, Multan, Bahawapur, D.G. Khan, Muzafargarh, Rahim Yar Khan and Lodhran. Whereas in 2004, relative occurrence was 29.1, 47.3, 60.5, 48.3, 35.6, 59.7, 51, 42, 48.7, 42.6, 48.5, 41.3, 32, 65, 38.4, 30.9, 42, 35.2, 25.6, 70.1, 51, 58.3, 63.4, 42.6, and 38.3% in Attock, Rawalpindi, Jehlum, Chakwal, Talagang, Gujranwala, Lahore, Okara, Sargodah, Mainwali, Sahiwal, Faisalabad, Jhang, Cheniot, Vehari, Sailkot, Narowal, Bhurewala, Khanewal, Multan,

Bahawapur, D.G. Khan, Muzafargarh, Rahim Yar Khan and Lodhran respectively (Fig. 5). In 2003, highest relative occurrence was recorded in Muzafargarh, Multan, Jehlum, Cheniot, Bahawalpur, Chakwal and Gujranwala but during 2004, highest relative occurrence was recorded in Multan followed by Cheniot, Muzafargarh, Jehlum, Gujranwala and D.G. Khan based on DAS-ELISA. Over all in the Punjab province, relative occurrence of ChiVMV recorded during 2003 and 2004 was 51.8 and 44.3% respectively (Fig. 2). No surveyed area was found free from ChiVMV infection; however, relative occurrence varied from one district to another.

One weed, identified as *Datura metel*, from *Capsicum* field (from districts of Chakwal and Mianwali) was found positive for ChiVMV infection while others such *Amarenthus viridis, Convolvulus arvensis* and *Albizzia lebbek* were found free from virus infection. The data shows that ChiVMV incidence was higher in mostly cultivated areas of the Punjab province and the trend is increasing from northern to southern part of the province. Similar observations have been reported earlier (Hameed *et al*., 1995; Shah & Khalid, 1999). The weed, *Datura metel* was also confirmed as host of ChiVMV through mechanical inoculation under glasshouse conditions. This weed may serves as an alternate host or source of inoculum foci for secondary infection in the subsequent season.

NWFP: In 2003, relative occurrence of ChiVMV recorded was 45, 44, 7, 3, 8, 3, 3, 8, 7, 9, 4, 1, 4 and 15 % in Malakand Agency, Swat, Charsadda, Mardan, Peshawar, Shinkyari, Haripur, Mansehra, Kohat, Karak, Bannu, Lakki Marwat, Hangue and D.I. Khan respectively but no infection was detected in samples from district Dir & Bajour Agency. In the subsequent year (2004), ChiVMV relative occurrence was 40, 38.7, 0, 0, 9.2, 6.5, 5.7, 3.5, 3.7, 9.4, 7.3, 10, 6.2, 0, 3.2 and 10.4% in Malakand Agency, Swat, Charsadda, Mardan, Peshawar, Shinkyari, Haripur, Mansehra, Kohat, Karak, Bannu, Lakki Marwat, Hangue and D.I. Khan except district Dir & Bajour Agency in the northern part and Lakky Marwat in southern part of the province (Fig. 6). Thus relative occurrence in the province was 41.3 percent without any significant change in 2003 and 2004 (Fig. 2). Samples from the weeds flora, viz., *Portulaca oleracea, Amaranthus viridis, Dactyloctenium sp. Eleusine sp., Echinochloa sp.*, and *Cyperus rotendus* were found free from ChiVMV infection.

During 2003 surveys, no ChiVMV was detected in district Dir and Bajour Agency but in the following year (2004) along with these two locations, Lakky Marwat in the southern part of the NWFP was also found free from ChiVMV infection. Similar findings about Lakky Marwat and some areas in Kohat district have been reported earlier (Shah & Khalid, 1999). One reason might be that these are less intensive growing areas of *Capsicum* and farmers might have grown for domestic use only on small-scale basis and thus no ChiVMV infection. However, in those areas where high relative occurrence of ChiVMV was encountered, three factors of disease development (virulent pathogens, susceptible host & conducive environment) were prevalent and thus high ChiVMV relative occurrence resulted. In Malakand Agency and district Swat, farmers mostly purchase seed from open local market for the last 4-6 years which seem hybrid as the seed germinability is low from collected from crop, farmers replied (Personal Communications). Marketable fruit picking from bell pepper continue before the on set of monsoon rain. But as the monsoon rain starts, flowering and fruit formation drop tremendously and the plants become diseased bearing no fruit or undersized/distorted fruit of less market value. Most of the samples collected were from either bell or hot type was found positive for ChiVMV from these two areas.

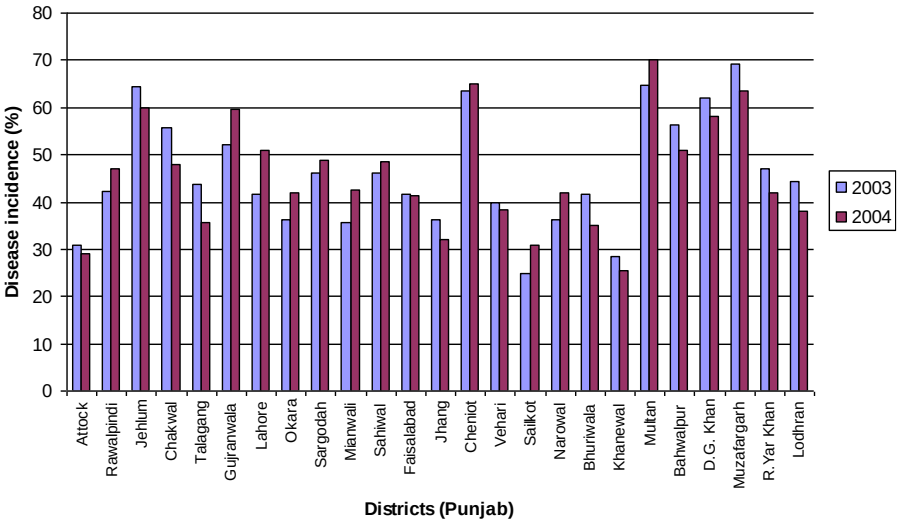

Fig. 5. Prevalence, distribution and relative occurrence of ChiVMV in major districts of Punjab during 2003 & 2004.

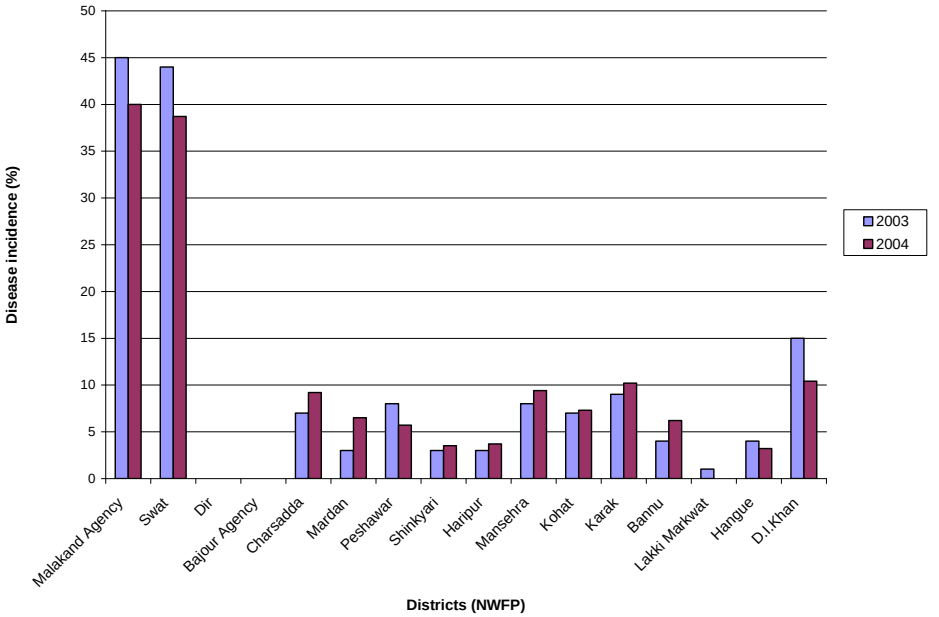

Fig. 6. Prevalence, distribution and relative occurrence of ChiVMV in major chili pepper growing districts of NWFP during 2003 and 2004.

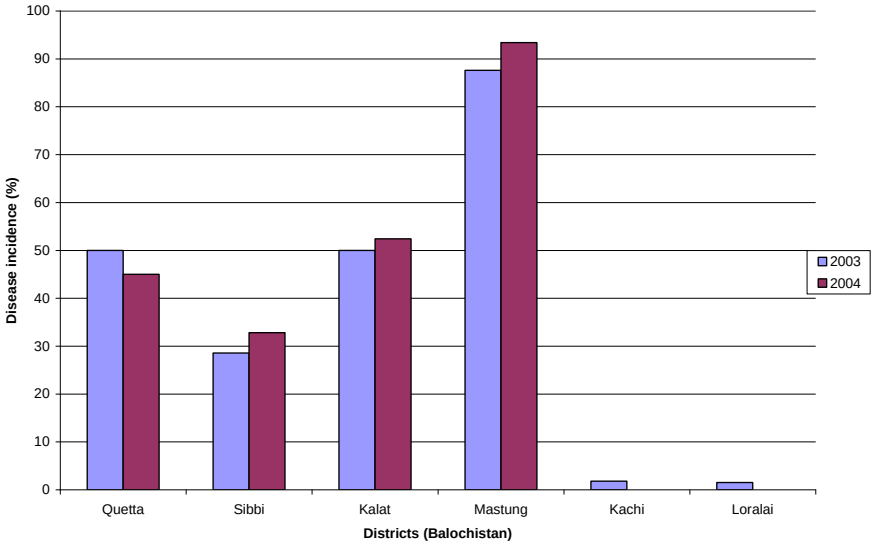

Fig. 7. Prevalence, distribution and relative occurrence of ChiVMV in major chili pepper growing districts of Balochistan during 2003 & 2004.

Balochistan: During 2003, the relative occurrence of ChiVMV was 50, 28.6, 50, 87.6, 1.8 and 1.5 % in Quetta, Sibbi, Kalat, Mastung, Kachi and Loralai respectively. In the following year of 2004, the relative occurrence of ChiVMV recorded was 45, 32.8, 52.4 and 93.4 % in Quetta, Sibbi, Kalat and Mastung respectively while no ChiVMV was detected in any samples from the other two districts i.e. Ziarat and Loralai of the province. Average relative occurrence was 38.7 and 34.5 % during 2003 and 2004 respectively and there is a slight decrease of 4% from previous year (2004). Highest relative occurrence was recorded in Mastung district followed by Quetta and Kalat while in Sibi district the relative occurrence was 28.6%. No virus was detected in any sample from districts of Kachi and Loralai during 2004 surveys (Fig. 7).

Discussion

The surveys of four provinces of Pakistan showed that all the commercially grown *Capsicum* genotypes were found susceptible to the ChiVMV infection. Such findings have been reported earlier in these provinces (Hameed *et al.,* 1995; Shah & Khalid, 1999). Co-infection of ChiVMV with other viruses, poses serious threat may be due to recombination and synergistic effect and its appearance in more devastated form is a constant threat under the prevailing cropping pattern and natural conditions. The gradual decline in production of chili pepper since 1998 is due to unknown reasons but most probably can be attributed to virus attack as the growers do not know the cause and switch to other crops. Moreover, the crop is least studied regarding pathological problems.

Comparing the relative occurrence of ChiVMV with previous surveys of 1993 and 1997, the relative occurrence of ChiVMV increased many fold as compared to the earlier surveys conducted during early 90s (Hameed *et al*., 1995). The cause of disease increase (19.6 %) might be due to a number of factors i.e., climatic conditions, low quality seeds,

alternate hosts and vector activity. In the earlier surveys mix infection (6-8 %) of four viruses (ChiVMV, CMV, TMV and PVY) was only detected in Sindh while co-infection of three viruses (ChiVMV, CMV and TMV) was 3.6 % and co-infection of two viruses (ChiVMV & CMV) was detected in 13 % of samples tested through DAS-ELISA (Hameed *et al.,* 1995; Shah & Khalid, 1999). A number of aphid species viz., *Myzus persicae* and *Aphis gossypii* have been reported from these districts of Sindh that transmit these viruses in non-persistent manner prevailing in high proportion on *Solanaceous* hosts in the province (Solangi *et al*., 1983). As chili pepper is grown in areas with warm day temperature and cool nights, therefore, such climatic conditions also favor vector's activity and might have aggravated the situation further. Moreover, general phyto-sanitary conditions were very poor and a large number of weeds were often found in and around chili field, possibly providing rearing niches for vectors as well as these viruses' foci. These infected fields might serve as a source of primary inoculum for ChiVMV in the next growing season.

Recent reports showed that aphid population has increased tremendously in the recent past due to favorable environmental conditions and might have transmitted ChiVMV to other Solanaceous hosts/weeds and has developed foci for secondary infection. The insect vectors of ChiVMV particularly aphids (*Myzus persicae* and *Aphis gossypii*) have broad host range (Solangi *et al*., 1983). The geographical distribution of ChiVMV is not absolute as its has been isolated sporadically in other parts of the world such as Eastern Africa (Brunt *et al.,* 1978; Nono-Womdin *et al.,* 2001) and Asia (Ong *et al*., 1979) where similar climatic conditions prevail as in Pakistan.

High relative occurrence of ChiVMV in Balochistan is obvious from the fact that these districts are adjacent at boundary of Jacobabad, Larkana and Dadu districts of Sindh province. But as the data has indicated that not a single location in these districts of Sindh was free from ChiVMV infection so occurrence in Quetta, Sibbi, Kalat and Mastung is the result of nursery importation from these ChiVMV infected districts. Thus, if nursery is infected, the subsequent crop raised from such nursery will be diseased and source of primary and secondary inoculum continue to persist in these districts. The farmers extensively grow chili pepper in their fields brought from Sindh province, which are major chili growing areas of the country.

As a routine the growers of Balochistan do not raise their own nurseries due to the climatic conditions of the province. The virus perpetuate from one growing season to another through such transportation of infected source. Further insect vectors might have aggregated its role in spread of the virus dispersal. It can not be ruled out that weed might be carrier of ChiVMV, such as *Datura metel,* found positive in samples collected from the province of Punjab. No such etiological studies have been conducted so far to find out the actual cause of primary inoculum of ChiVMV leading to high relative occurrence in these districts. Weeds may act as reservoirs of virus or its vectors within the crop and in areas adjacent to crop plants.

Virus infected weeds within crop are a serious threat because of the close proximity of the source of primary inoculum to the healthy ones (Duffus, 1971). Control can be achieved by elimination of the weeds by rouging and/or by using herbicides. However, viruses may persist in these weeds. Some viruses such CMV and Nepoviruses have been reported that spread through seeds of annual weed i.e., *Stellaria media* (Chickweed) (Stevens, 1983). Perennial weeds may act as source of viruses in diseases of legumes and cucurbits. Nearly wild and garden source of infection are important in the epidemiology of virus diseases pepper (Thresh, 1976). Wild plants have long been presumed to

contribute to virus spread and relative occurrence. Viruses come from sources of infection, which may be other than main crops or closely related crops.

The symptoms observed during these surveys are similar to the findings of Ong *et al.,* (1979) who reported that vein mottling were more pronounced on bell type than on hot types. Variability in symptoms i.e., vein mottling, malformation, reduction in leaf areas were continuously observed surveys due to differences in cultivars, time of infection and climatic conditions which have been reported by Abu-Kasim (1986). Previously, similar field symptoms on both types have been reported by Fujisawa *et al.,* (1986) in Japan, Hameed *et al.,* (1995) in Pakistan, Prakash *et al.,* (2002) in India; and Cerkauskas *et al*., (2004) in Taiwan, Republic of China.

Prakash *et al.,* (2002) reported relative occurrence of ChiVMV in the range of 5-75% with an average of 50% in India, which is close to the incidence (44%) recorded in Pakistan during 2003 and 2004. It is well known fact that environmental conditions of both countries are similar and agronomic practices are the same with the same soil characteristics and thus experienced similar incidence of ChiVMV. Moury *et al.,* (2005) reported that the ChiVMV clade comprises three subgroups i.e., the East Asian ChiVMV isolates, Ethiopian and Yemeni PVMV isolates, and the west and central African PVMV isolates, which correspond to the groups obtained with pairwise similarities and it is possible that geographic isolation could be responsible for diversification within this group. It is further speculated that specificity of resistance only partially corresponded to molecular diversity of the isolates and has suggested that much variability exists within ChiVMV and appearance of virulent isolates is a rare event or virulent isolates are less fit and do not spread efficiently in the agro-ecosystem. However, the evolutionary forces that drove speciation within the group are complex yet unknown but need to be explored.

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