Research Article

Incidence and distribution of begomoviruses (BGMV) infecting ornamental plants in Multan Region, Pakistan

Faheem Zia[1], Hasan Riaz*[1], Muhammad Ashfaq[1], Sufyan Raza [1], Sabeeh Khan[1], Seema Kanwal [1], Muhammad Hassan[1], Muhammad Ishtiaq[1], Muhammad Shahzad Zafar[2], Muhammad Imran[3], Sarmad Frogh Arshad[4], Saman Shafiq[1]

[1] *Institute of Plant Protection, Muhammad Nawaz Shareef University of Agriculture, Multan*
[2] *Mango Research Institute, Multan, Pakistan*
[3] *Department of Soil & Environmental Sciences, Muhammad Nawaz Shareef University of Agriculture, Multan*
[4] *Institute of Plant Breeding and Biotechnology, Muhammad Nawaz Shareef University of Agriculture, Multan*

* Correspondence: hasan.riaz@mnsuam.edu.pk

Abstract: Begomovirus is the largest and important genus in virus taxonomy. It has more than 400 widespread species causing diseases in different plant families. Besides cash crops, begomoviruses also infect ornamental plants which lead to significant losses and lower market value. The accurate and precise knowledge of incidence and distribution of the plant viruses is mandatory for the management of plant viruses. The research study was aimed to assess the distribution and to estimate the disease incidence of begomoviruses infecting ornamental plants in Multan region which include district Multan, Lodhran, Khanewal and Vehri. Overall, 174 leaf samples of ornamental plants from different sites in Multan region were collected. Total genomic DNA from ornamental plants were extracted with CTAB method and subjected to PCR for the amplification of partial coat proteins gene using universal primers. Total incidence data of begomoviruses infecting ornamental plants in Multan region was calculated as 54% while district wise disease incidence percentage was recorded as 58.06% in district Multan, 55.88% in Vehari, 55.26% in Lodhran and 50% in Khanewal. High disease incidence was recorded in *Euphorbia tithymalioides* as 77.7%, followed by *Hibiscus rosa-sinensis* 71.4%, *Euphorbia milli* 70.5%, *Tabernaemontana divaricate* 66.6%, *Tecoma stans* 54.5%, *Jasminum sambac* 37.5% and *Radermachera sinica* 20% while *Quisqualis indica*, *Irresine herbstii*, *Cordia dichotoma*, *Ficus benjamina*, *Bougainvillea* and *Hamelia patens* were found uninfected. Uneven distribution of begomoviruses was observed with maximum distribution 38.2% in district Multan and minimum 19.1% in Vehari. The study gave a vivid picture of ornamental plants harboring begomoviruses and that could lead to a vital part of an integrated management of these viruses.

Keywords: Begomovirus, PCR, Ornamental Plants, Infection, Disease Incidence

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1. Introduction

Crop plant cultivation has been constantly remained praiseworthy for food production but people have never ignored the ornamental plants. The garden culture was adopted by Persians since the antiquity, the word paradise is actually originated from Persian term pairidaeza. Roses were grown in China about 5,000 years ago. In ancient times (2800BC) the ornamental plants like dwarf palm (*Chamaerops humilis*), blue lotus (*Nymphae caerulea*) and papyrus (*Cyperus papyrus*) were used by the Egyptians to decorate buildings and places of worship. Religious texts also emphasized the importance of nature preservation; hence, muslim civilization has accorded significant reverence to nature. That is why the muslim botanist has achieved such prominence and notoriety: Around 1080 AD, Ibn Bassal authored a book on the cultivation of garden plants [1].

Different ornamental plants remain in the landscapes throughout the year. However, these ornamentals may act as host of viral pathogens providing safe heaven for recombination and play a significant role in transmission. Among the viral infection in plants begomovirus has considerable relevance as it the largest genus in virus taxonomy. The plant disease caused by begomovirus is the earliest viral disease reported in history. A Japanese poetess Empress koken narrated a plant showing yellow leaf color in her poem in 752 AD which was translated in English by T. Inouye. Eupatorium lindleyanum was a plant that was showing yellowing of leaf instead green colour [2]. It is believed that the plants of Eupatorium that were exhibiting the symptoms of yellow colour were infected with begomovirus infection, Eupatorium infecting begomoviruses were named as Eupatorium yellow vein virus and Eupatorium yellow vein betasatellite [3].

Begomoviruses are whitefly persistent manner transmitted plant viruses having monopartite (ssDNA circular molecule of approximately 2.7 kb) or bipartite genomes (two circular ssDNA molecules, each about 2.6 kb) are found in both New World (mostly bipartite genomes) and Old World (both genome types) and infect dicot plants [4]. Begomoviruses are economically most destructive inducing vein thickening, severe leaf curling, enation and symptoms of mosaic pattern in their host plants and are harmful among the viruses of geminiviridae family. Begomoviruses have emerged as serious plant infecting viruses invading ornamental plants along with food and fiber crops grown on a large area of the world over the last 30 years [5, 6]. The present study was conducted to determine the incidence distribution of begomoviruses infecting the ornamental plants in Multan region and their distribution in four districts of Multan region through polymerase chain reaction.

2. Materials and Methods

A total of forty thirty-eight plant nurseries where various kinds of ornamental plants were present were visited during 2019-2021 in four districts of Multan region, viz Khanewal, Vehari, Lodhran and Multan. Ornamental plant leaf samples with symptoms such as yellowing, mosaic, vein thickening, vein yellowing and stunting along with asymptomatic plant leaf samples were collected through random sampling. A total of 174 samples were collected, kept in polythene bags in an ice bucket, and brought to the lab for further processing. To remove any surface contaminants or contamination, the samples were rinsed with distilled water and stored at -80° C.

Total genomic DNA extraction and quantification

Total DNA was extracted from ornamental plant samples by modified CTAB method [7]. In the presence of liquid nitrogen, 0.1 g of plant leaf sample was ground to a fine powder in a pestle and mortar followed by addition of 700µl pre-heated (Heated at 65 °C for 30 minutes) CTAB buffer. Ground leaf sample was shifted into 1.5ml sterile microcentrifuge tube and these eppendorf tubes were kept in water-bath for 30 minutes at 65 °C. After cooling down, equal volume of chloroform: isoamyl alcohol (24:1) was added to 1.5 ml micro centrifuge tube and gently mixed. Mixture containing Eppendorf tubes were centrifuged at 12000rpm for ten minutes at 4 °C. The aqueous phase (supernatant) was shifted into new eppendorf tube and 0.6 % volume of chilled isopropanol was added. Eppendorf tubes were incubated on ice for 20 minutes or incubated overnight for DNA precipitation at room temperature. Eppendorf mixture was centrifuged at 12000rpm for 3 minutes. Supernatant was discarded. Pellet was washed once with 500µl absolute ethanol and twice with 500µl 70% ethanol. When 70% ethanol was used to wash the pellet, the pellet was not vortexed, because 70% ethanol has 30 % water and this 30% water is enough to dissolve this pellet and laborious to recover. After washing, the pellet was dehydrated, then it was dissolved in 100-200µl double distilled sterilized water. The integrity of the extracted DNA was checked on 1% agarose gel prepared in 1xTAE buffer and stored at 80 °C. Quantification of DNA was done by using nanodrop. The DNA integrity was recorded by observing the A260/A280 ratio and wave length(nm).

Molecular detection of begomoviruses

To validate the presence of begomoviruses molecular identification of DNA-A was done by nucleic based technique (PCR), based on partial coat protein gene primer pair. Selected DNA (having A260/A280 ratio of 1.7 – 2.0 ng/µl in nanodrop) samples were subjected to polymerase chain reaction (PCR) to check the begomovirus infection. Nuclease free water (15.0 µL), DNA (1.0 µL), dNTPs (1.0 µL), PCR Buffer (2.5µL), MgCl2 (2.5 µL), TB forward primer (1.25 µL), TB reverse primer (1.25 µL), and DNA taq polymerase (0.5 µL) was taken in a PCR tube and this PCR tube was placed in a PCR machine (96 well thermocycler) to carry out the polymerase chain reaction. Conditions for PCR were set as initial denaturation at 96 °C for 5 minutes and 1 cycle, denaturation at 96°C for 1 minute and 30 total passes, annealing at 57°C for 30 seconds and 30 passes, extension at 72°C for 30 seconds and 30 passes and final extension at 72°C for 10 minutes and 1 cycle. The begomoviruses incidence was recorded by using the formula given below

$$\text{D.I \%} = \frac{\text{Number of infected samples}}{\text{Number of tested samples}} \times 100$$

3. Results

The Multan region including four districts viz. Vehari, Khanewal, Lodhran and Multan were surveyed during 2019-2021 and overall, 174 symptomatic along with asymptomatic leaf samples (figure 1) from various ornamental plants were collected. Plant leaf samples were collected from different sites, after capturing the pictures of these samples they were brought to lab in an ice containing box and stored at -80° C. All 174 samples were subjected to PCR using begomovirus specific degenerate primers for the validation of begomoviruses infection. The incidence of begomoviruses in Multan region was high i.e., 54.02% however maximum disease incidence was recorded in Multan (58.06 %) followed by Vehari (55.88%), Lodhran (55.26%) and Khanewal (50%) (figure 3-8). Moreover, plant wise disease incidence was also calculated as highest disease incidence was observed in Euphorbia tithymaloides (77.7%) followed by Hibiscus rosa-sinensis (71.42%), Cestrum nocturnum (70.58%), Euphorbia milli (70.58%), Tabernaemontana divaricate (66.6), Tecoma stans (54.5%), Jasminum sambac (37.5%) and Radermchera sinica (20%). while, Quisqualis indica, Cordia dichotoma, iresine herbstii Ficus benjamina, Bougainvillea, jatropha integrrima and Hamelia patens were found uninfected (figure 2). Distribution of begomoviruses was calculated using the formula

$$\text{Begomoviruses distribution} = \frac{\text{Number of infected samples in a district}}{\text{Total infected samples in region}} \times 100$$

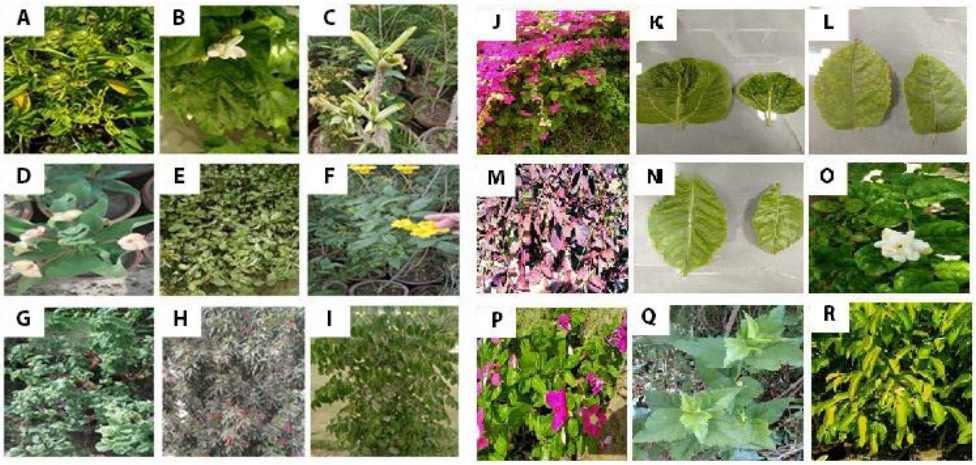

Figure 1: Ornamental plants collected to check begomovirus infection (A) *Cestrum nocturnum* (B) *Jasminum sambac* (C &D) *Euphorbia milli* (E) *Euphorbia tithymaloides* (F) *Tecoma stans* (G) *Mentha*

piperita (H) *Jatropha integrima* (I) *Radermachera sinica* (J) *Bougainvillea glabra* (K) *Cordia dichotoma* (L) *Gardenia* (M) *Irresine herbstii* (N) *Santalum album* (0) *Jasmine* (P) *Catharanthus roseus* (Q) *Laportea peduncularis* (R) *Ficus nitida*

Table: 1. PCR results of collected samples

	Host Name	Symptoms	PCR Results
A.	***Hibiscus rosasinensis***	Severe curling and yellowing of leaves; vein thickening	Positive
B.	***Euphorbia milli***	Leaf curling and vein thickening	Positive
C.	***Jasminum sambac***	Severe leaf curling and vein thickening	Positive
D.	***Quisqualis indica***	No symptoms	Negative
E.	***Tabernaemontana divaricate***	No clear symptoms	Positive
F.	***Cordia dichotoma***	No symptoms	Negative
G.	***Iresine herbstii***	Leaf curling and vein thickening	Negative
H.	***Ficus benjamina***	No Symptoms	Negative
I.	***Radermachera sinica***	Mosaic like symptoms	Positive
J.	***Euphorbia tithymaloides***	Severe leaf curling with severe vein thickening	Positive
K.	***Cestrum nocturnum***	Severe leaf curling, severe vein thickening and leaf yellowing	Positive
L.	***Bougainvillea***	No clear symptoms	Negative
M.	***Tecoma stans***	Mild vein thickening	Positive
N.	***Catharanthus roseus***	No symptoms	Negative
O.	***Jatropha integerrima***	No symptoms	Negative
P.	***Hamelia patens***	No symptoms	Negative

S. No.	Plant	Multan		Lodhran		Khanewal		Vehari		Total	
		Infected	Total	Infected	Total	Infected	Total	Infected	Total	Infected	Total
1	***Hibiscus rosa-sinensis***	2	3	5	7	5	7	3	4	15	21
2	***Euphorbia milli***	3	3	2	3	3	5	4	6	12	17
3	***Jasminum sambac***	7	8	6	7	5	6	1	3	19	24
4	***Quisqualis indica***	0	1	0	0	0	1	0	0	0	2
5	***Tabernaemontana divaricate***	5	6	2	3	0	1	1	2	8	12
6	***Cordia dichotoma***	0	1	0	0	0	0	0	0	0	1
7	***Iresine herbstii***	0	2	0	2	0	1	0	3	0	8
8	***Ficus benjamina***	0	1	0	2	0	0	0	0	0	3
9	***Radermachera sinica***	0	2	0	0	1	2	0	1	1	5
10	***Euphorbia tithymaloides***	9	11	4	6	2	3	6	7	21	27
11	***Cestrum nocturnum***	6	8	2	3	2	3	2	3	12	17
12	***Bougainvillea***	0	1	0	0	0	1	0	0	0	2
13	***Tecoma stans***	4	6	0	0	0	2	2	3	6	11
14	***Vinca rosea***	0	2	0	2	0	3	0	0	0	11
15	***Jatropha integerrima***	0	5	0	3	0	0	0	2	0	10
16	***Hamelia patens***	0	2	0	0	0	1	0	0	0	3
	Disease Incidence	**58.06452**		**55.26316**		**50**		**55.88235**		**54.02299**	

Figure 2. Disease incidence of ornamental plants

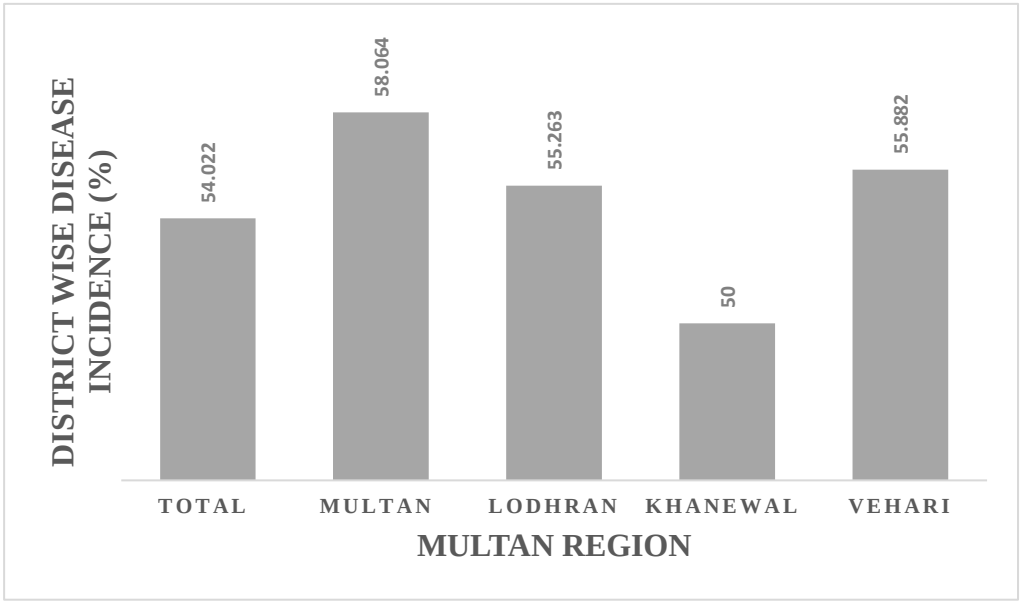

Figure 3: Disease incidence in Multan region

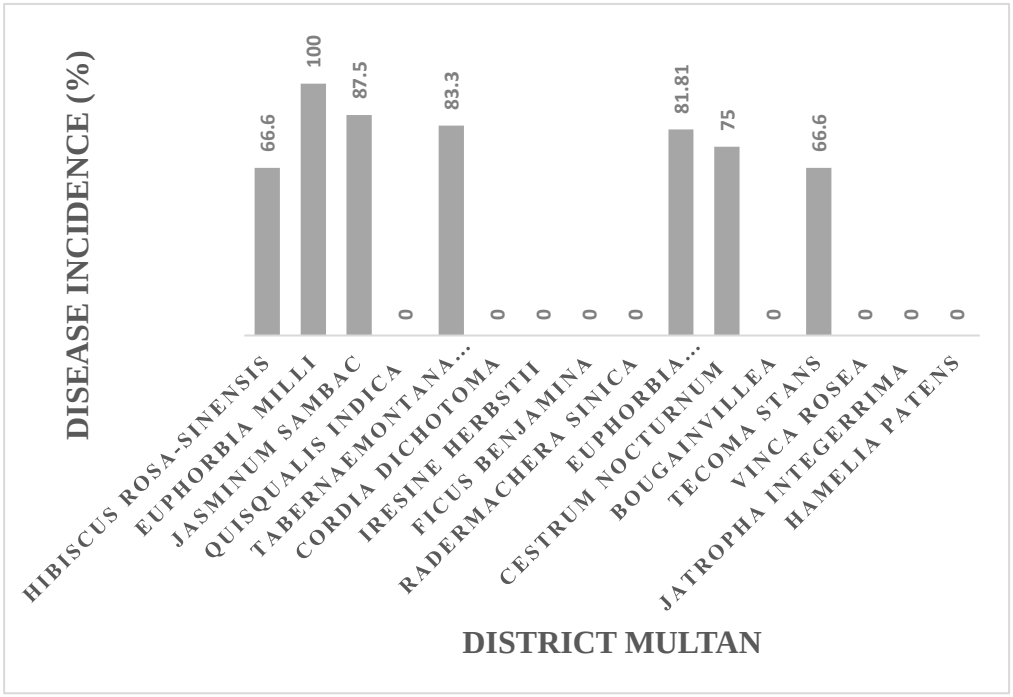

Figure 4: Disease incidence in district Multan

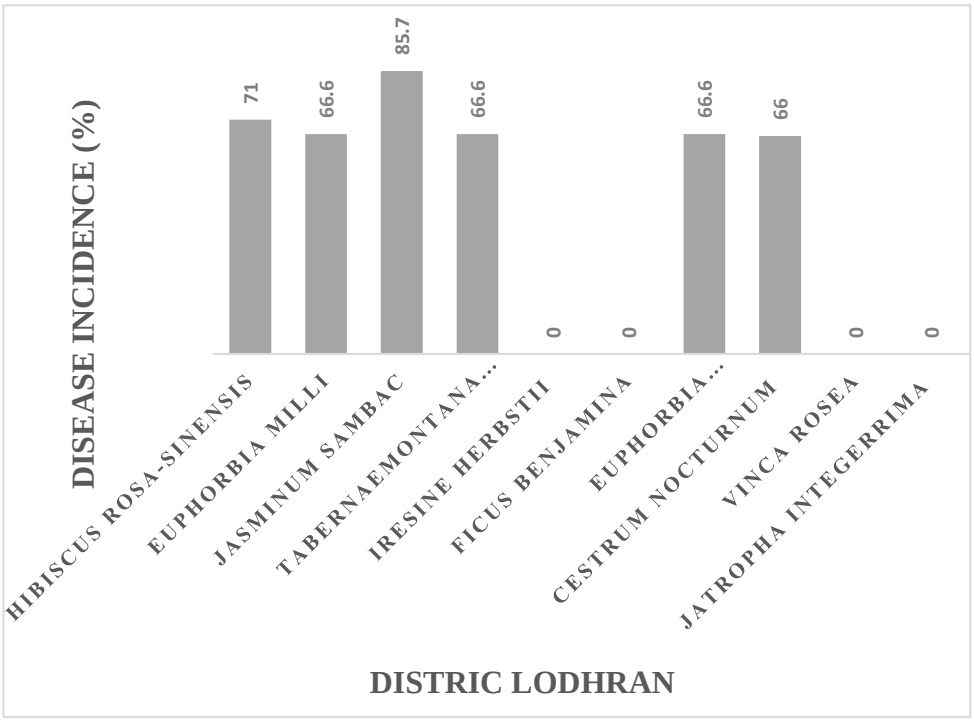

Figure 5: Disease incidence in district Lodhran

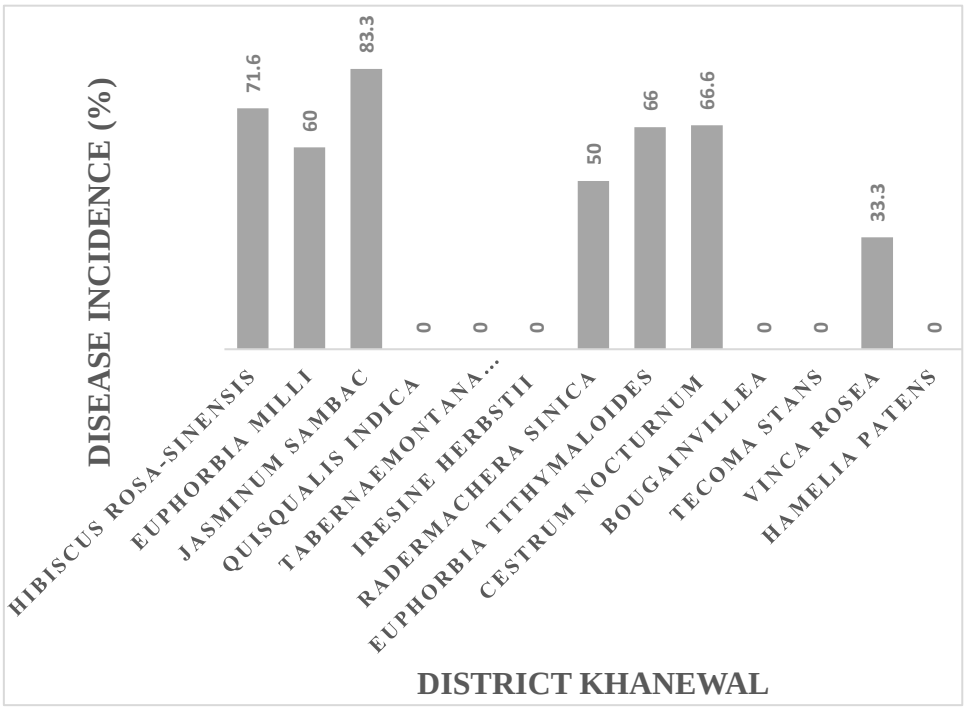

Figure 6: Disease incidence in district Khanewal

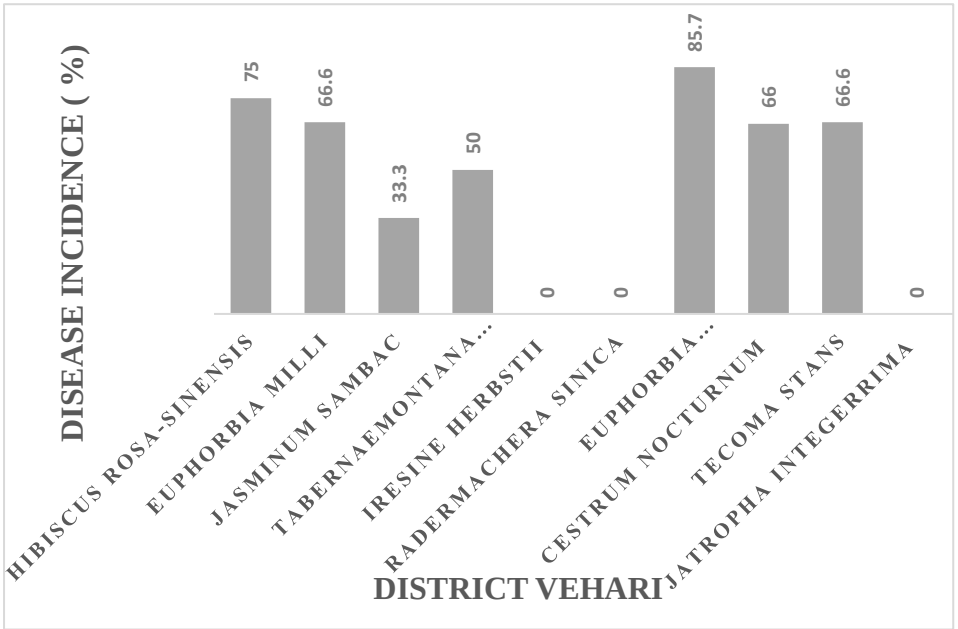

Figure 7: Disease incidence in district Vehari

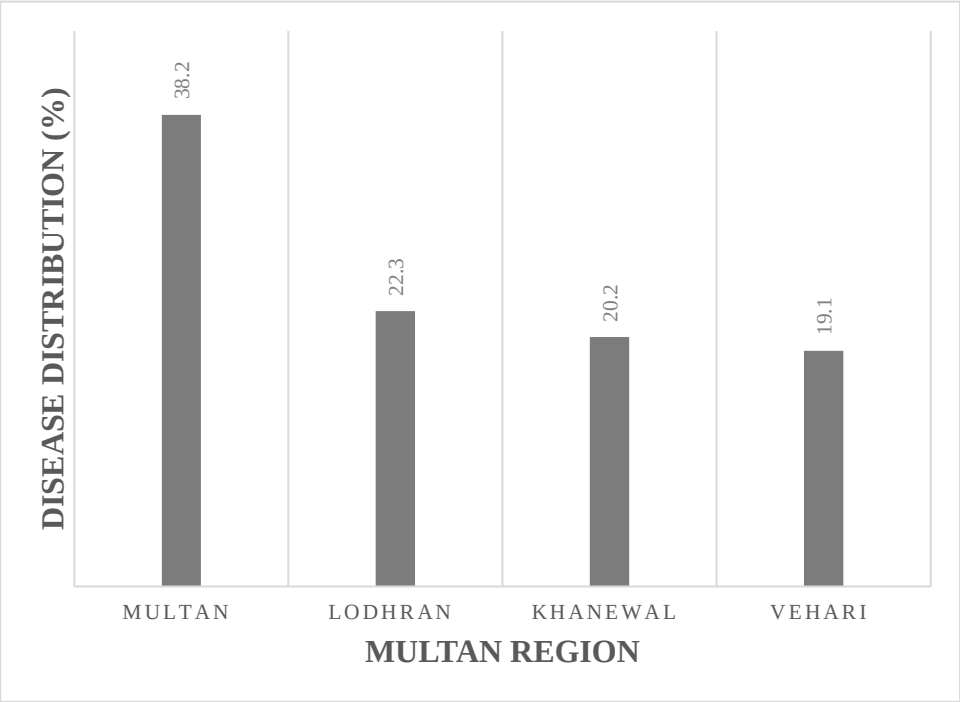

Figure 8: Begomoviruses distribution in Multan region

4. Discussion

The begomoviruses are vectored by whitefly in a persistent manner. Whitefly efficiently circulates the begomoviruses which are infecting economically significant plants like, vegetables, field crops and ornamentals worldwide [8]. Generally, *Bemisia tabaci* makes begomoviruses transmission successful in a persistent manner, but some begomoviruses like; SPLCV, TLCYV and MYMV are transmitted through seed [9,10].

In Multan region due to diversification of cultivated plants and favorable conditions for whitefly lead to high incidence of begomviruses. All four districts of Multan region were surveyed to observe the begomoviruses infection in ornamental plants, this study shows that there is no single district free of begomoviruses infection in Multan region. All the symptoms which are observed during the survey are also reported previously [11]. During this research 2019-2021, plants of Euphorbeaceae family found infected with begomoviruses and such type of conclusions are previously reported [12]. The present research work confirms the begomoviruses infection in *Tabernaemontana divaricate*, which is in line with the findings of [13] who reported Bhendi Yellow Vein Mosaic Virus (BYVMV) infecting *Tabernaemontana divaricate* in India. *Tecoma stans* found to be infected with begomoviruses infection in this study and these types of findings are also previously observed [14]. Begomoviruses are also found to infect *Hibiscus rosa sinensis*, which is in harmony with earlier reports [15, 16]. *Jasminum sambac* gave positive results on PCR which confirms the begomoviruses infection which is already found infected with begomoviruses in different countries of the world [17,18]. *Quisqualis indica*, *Cordia dichotoma*, *Ficus benjamina*, *Rdermachera sinica*, *Bougain villea* and *Hamelia paten* gave negative results upon PCR analysis. In case of *Jatropha integrrima* and *Bougain villea*, the present research work is contrary to previous findings [19, 20].

Begomoviruses have been found infecting the variety of ornamental plants like *Euphorbia tithymaloides*, *Hibiscus rosa-sinensis* and *Jasminum sambac*. PeLCV in association with Tobacco leaf curl betasatellite was first isolated from *Pedilanthus tithymaloides* in 2009 from Pakistan [20] has rapidly increased its host range in Pakistan. There have also been reports of the PeLCV-TbLCuB complex, infecting *Glycine max* [21], *Sesbania bispinosa* and *Raphanus*

sativus indicating that the virus host range is constantly expanding [22, 23]. As begomoviruses have devastating effects on various cultivated plants and harbouring in ornamental plants which might lead to new severe variants.

5. Conclusions

The present study shows the distribution and incidence of begomviruses infecting ornamental plants in Multan region in a comprehensive manner. These results lead to source of understanding for the study of begomoviruses infection. Therefore, there is an extreme need to make strategies to control these distressing plant viruses.

Conflicts of Interest: All the authors have no conflict of interest.

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