

Research Article

Incidence and distribution of *Zucchini yellow mosaic virus* (ZYMV) infecting Cucumber (*Cucumis sativus* L) crop in Pothowar, Pakistan

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Citation

Zohaib Asad, Muhammad Ashfaq, Tariq Mukhtar and Muhammad Tariq. Incidence and distribution of *Zucchini yellow mosaic virus* (ZYMV) infecting Cucumber (*Cucumis sativus* L) crop in Pothowar, Pakistan. Pure and Applied Biology. Vol. 8, Issue 3, pp2036-2043. <http://dx.doi.org/10.19045/bspab.2019.80148>

Received: 02/05/2019

Revised: 15/07/2019

Accepted: 23/07/2019

Online First: 26/07/2019

Abstract

Zucchini yellow mosaic virus (ZYMV) is one of the most destructive virus of cucurbits and found throughout the cucurbits growing areas of Pothowar region of Pakistan. Overall 300 samples were randomly collected from 40 cucumber growing fields from all over the pothowar region during two consecutive years (2015-16 and 2016-17). All the samples were screened by Double Antibody Sandwich- Enzyme Linked Immunosorbant Assay (DAS-ELISA). Relative incidence of ZYMV recorded in two consecutive years was 60.6% and 66% respectively. The highest disease incidence of ZYMV was 77% during 2015-16 in Chakwal while during 2016-17 highest disease incidence was 71% recorded from Rawalpindi. No district of pothowar region was found to free from viral infection. Among these samples weed flora was also collected where available and subjected to DAS-ELISA for conformation as alternate host of virus. Reaction plants were also used during experiment for virus conformation and for pathogenicity test. The core interest of this study was to explore the incidence of ZYMV infecting cucumber in pothowar region through serological assay.

Keywords: ELISA; Incidence; Pothowar; ZYMV

Introduction

Cucumber (*Cucumis sativa* L) is one of most important crops belongs to family *Cucurbitaceae* and for over 3,000 years cultivated by man [1, 2]. It is a soft, succulent plant having high content of water and has large canopy of leaves which covers the fruit. The vines are grown on trellises or stakes. The fruit is roughly cylindrical, elongated having tapered ends, mostly used in unripe condition, usually

eaten as salads or pickled and are in tropical region also stewed [3].

As for monetary importance, it has fourth positions after tomatoes, cabbage and onion in Asian continent [4] and in Western Europe, it is second most important vegetable crop after tomato [5]. Cucumber is enriched with nutrients and it has also contained, vitamin C, thiamine niacin, phosphorous, iron, calcium. The low production is hampered by biotic and abiotic factors and unavailability of

resistant varieties. Among the biotic factors viral infection is a standout amongst the most critical reasons of ailment [6]. Among these viruses, *Zucchini yellow mosaic virus* (ZYMV) is considered as a major yield-reducing agent in cucumber crop. Most prominent symptoms produced by ZYMV on leaf are mosaic, blistering, and size of leaf became reduced. Infected plants are stunted. Fruit symptoms encompass knobby areas which cause embossed deformation, and irregular skin coloring [7]. *Zucchini yellow mosaic virus* (ZYMV) is an important member of the genus *Potyvirus* in the family *Potyviridae* [8] and was first reported in 1973 in Italy [9]. The virus spread worldwide within a decade, resulting in significant economic losses and yields reduction of cucurbit crops [7]. Number of aphid species are responsible for transmitting ZYMV in a non-persistent manner [10, 11]. In some cucurbit crops ZYMV is also transmitted through seed transmission at very low rates [8, 12]. Weed hosts act as virus reservoir and function as a primary source of inoculum which leads to the development of disease epidemics [13]. According to Riedle-Bauer [14], wounds produced during mechanical weeding operations also enhance the transmission of ZYMV from plant-to-plant and it may also be carried out by vertebrates like rabbits. However, there is no experimental evidence to favor these ideas. Diseases caused by

plant viruses can be controlled by the use of highly resistant varieties and by the use of advanced techniques like nanotechnology. Present study was conducted to evaluate the prevalence and distribution of ZYMV in the Pothwar region by enzyme-linked immunosorbent assay (ELISA).

Materials and methods

Overall forty cucumber fields were randomly visited in the summer season during 2015-16 and 2016-17 in four districts of the Pothwar region, viz Rawalpindi, Attock, Chakwal, Jhelum and capital territory (Fig. 1). Random sampling was done in such a way that leaf and fruit samples of cucumber exhibiting symptoms like yellow mosaic, necrosis, blister, distortion, fan-leaf appearance, shoestring, stunting were collected. Overall 300 samples were collected. Weeds (*Datura* spp, *Kulfa*, (*Portulaca oleracea*) *Deela* (*Cyprus rotundus*) were also collected from cucumber fields where they were available and subjected to DAS-ELISA to investigate as an alternate host of ZYMV in the fields. Along with weed flora some reaction plants are also tested for virus confirmation and symptoms development. However, in calculating the incidence data of the virus, they were not considered. All samples collected were placed in polythene bags, stored at 4°C and subjected to DAS-ELISA for virus confirmation.



Figure 1. Map showing the prevalence of ZYMV in Pothwar region

Serological assay

Collected samples were subjected to DAS-ELISA (Double Antibody Sandwich-ELISA) as performed [15] for investigation of virus from infected cucumber leaves collected from different localities of pothowar region. Polystyrene plates were coated with anti ZYMV antibodies (Bioreba AG, Switzerland), diluted 1:200 in coating buffer and incubated overnight at 4°C. Saps of infected leaves were extracted by using extraction buffer in mortar with pestle and filtration was done through double layer of muslin cloth. 200µl of the filtered sap of each sample was taken and then put into the coated polystyrene plate followed by incubation overnight at 4°C. Alkaline phosphatase-conjugated anti-ZYMV antibodies (Bioreba AG) were added and incubated overnight at 4°C, after that incubation with p-nitrophenyl phosphate (MP Biomedicals, Inc. Ohio, USA) is done at room temperature for 1 h. Automatic ELISA Reader (HER-480 HT Company (Illford) Ltd., UK) is used to measure absorbance values (405 nm). Samples were considered significantly positive for ZYMV infection when the ELISA absorbance value was twice or higher than the average absorbance value of the healthy tissue as well as negative control. ZYMV ELISA kit was equipped with commercial positive and negative controls (Bioreba).

Bioassay

Biological characterization and Pathogenicity test were carried out by mechanical inoculation and through aphid transmission. For this purpose, in 0.05 M phosphate buffer, pH 7.2, containing 1% Na₂SO₃, the tissue of young leaves or fruit (1/3 w/v) with typical symptoms was homogenized as described by Ashfaq *et al.* (2010). Bioassay was conducted on the following test plants. (*Chenopodium amaranticolor*, *C. quinoa*, *Nicotiana tabacum*, *Cucumis sativus cv*, *Capsicum annum cv*, *Datura stramonium*, *Luffa cylindrica*, *Cucurbita moshata*, *Cucumis melo*, *Pasium sativum* in control

greenhouse conditions. Symptoms development was investigated post inoculation after every two days up to one month.

Results

Total 40 fields were surveyed, and 300 plant samples were randomly collected during survey process in two consecutive years 2015-16 and 2016-17. All the samples collected, were subjected to DAS-ELISA and results of serological test showed that there was no pothowar district found free of ZYMV prevalence. Relative incidence of ZYMV was recorded in two consecutive years was 60.6% and 66% respectively as shown. (Fig. 2)

ZYMV prevalence during 2015-16 in pothowar region

Mostly cucumber crop is growing in all district of pothowar region and capital territory Islamabad throughout the season. During survey relative incidence of ZYMV was 66%, 65%, 50%, 77%, 57% recorded in Islamabad, Rawalpindi, Attock, Chakwal, and Jhelum respectively. The highest disease incidence was recorded 77% in Chakwal. (Fig. 3). During survey it is noted that ZYMV was present in all over the pothowar region. The basic reason of its persistence is its inoculum which remains in the fields and shifts from one location to another due to the transportation of infested materials by human activities whereas for the long range spreading of inoculum is caused by viruliferous aphids through wind to a new cultivated area. During survey aphid populations are also observed on infected plant. Unluckily, farmer in this region are not aware of how viruses are dispersing from one plant to another and do not know about management strategies to control virus transmission. It is also noted that favorable environmental conditions also aggravate aphid population.

ZYMV prevalence during 2016-17 in pothowar region

During 2016-17 highest disease incidence was recorded as 71% in Rawalpindi followed by 68% in Chakwal 64% in Islamabad, 58% in Attock as well as in

Jhelum. (Fig. 4). During this year disease incidence increased in most of the areas of pothwar region. Increase in disease incidence is attributed to climatic conditions throughout the year. Day by day increase in temperature helps the virus vector (aphids) to proliferate and transmit the virus more rapidly.

Comparison of ZYMV prevalence in different areas of pothwar region during two consecutive year

Results shows that incidence of ZYMV mostly increase during 2016 as compare to 2015 in some districts. (Fig. 5) Increase in disease incidence is attributed to climatic conditions throughout the year. Day by day increase in temperature helps the virus vector (aphids) to proliferate and transmit the virus more rapidly. It is also attributed to lack resistant varieties as well as poor management practices by the farmer and dis-functionality of agriculture extension department.

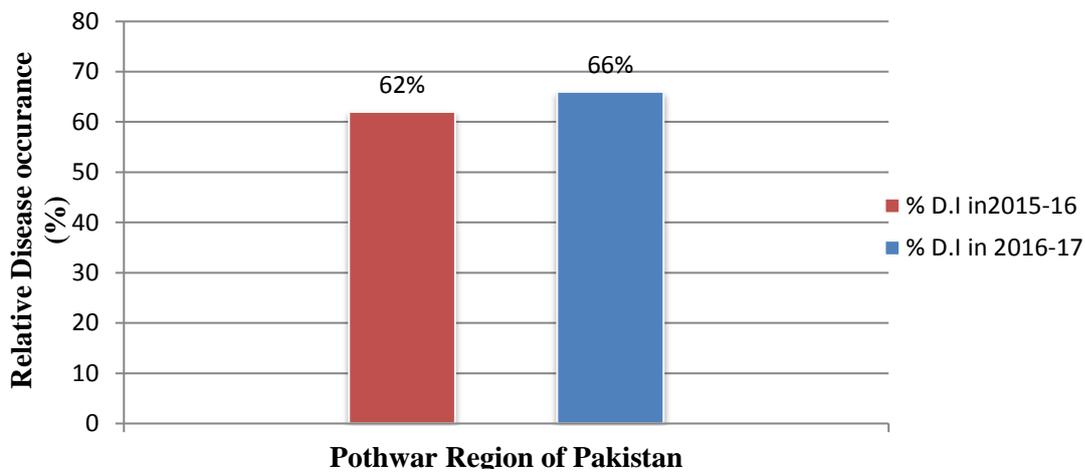


Figure 2. Relative Disease occurrence (%) during 2015-16 and 2016-17

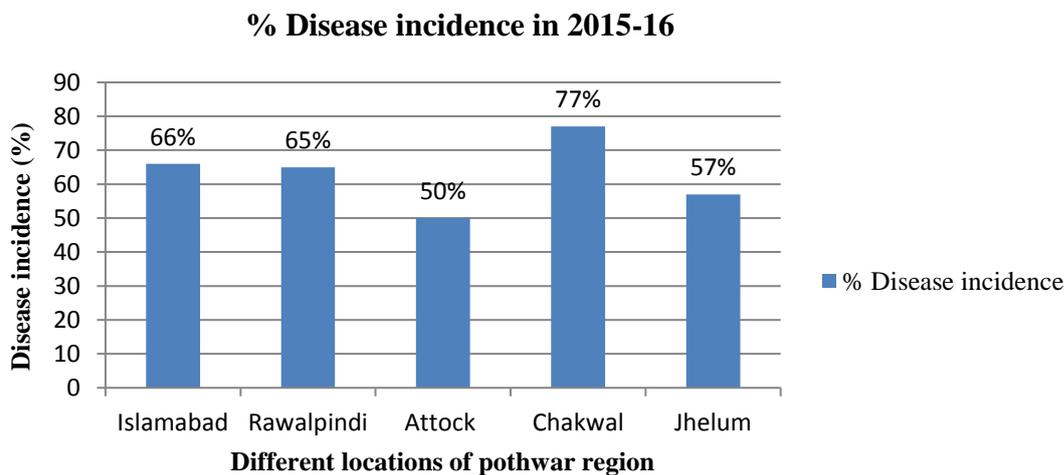


Figure 3. Disease incidence (%) of ZYMV in different areas of pothwar region during 2015-16

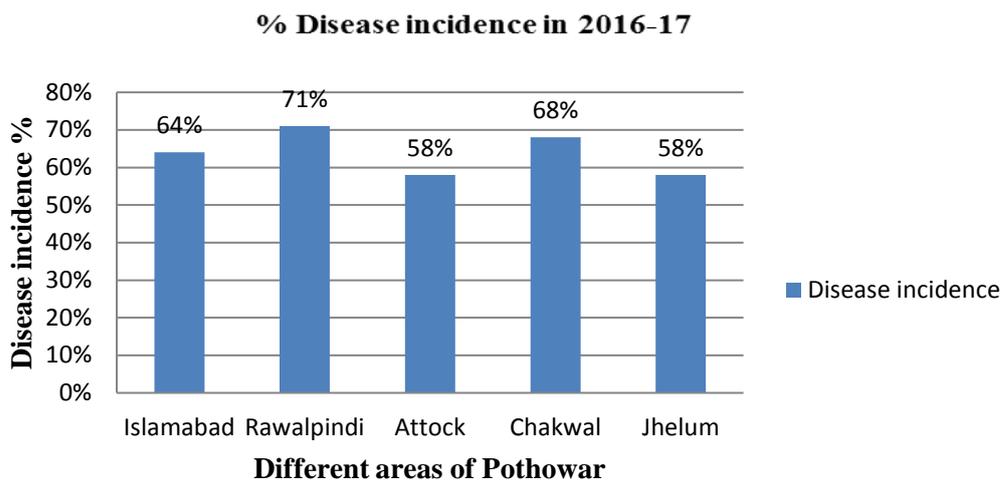


Figure 4. Disease incidence (%) of ZYMV in different areas of pothwar region during 2016-17

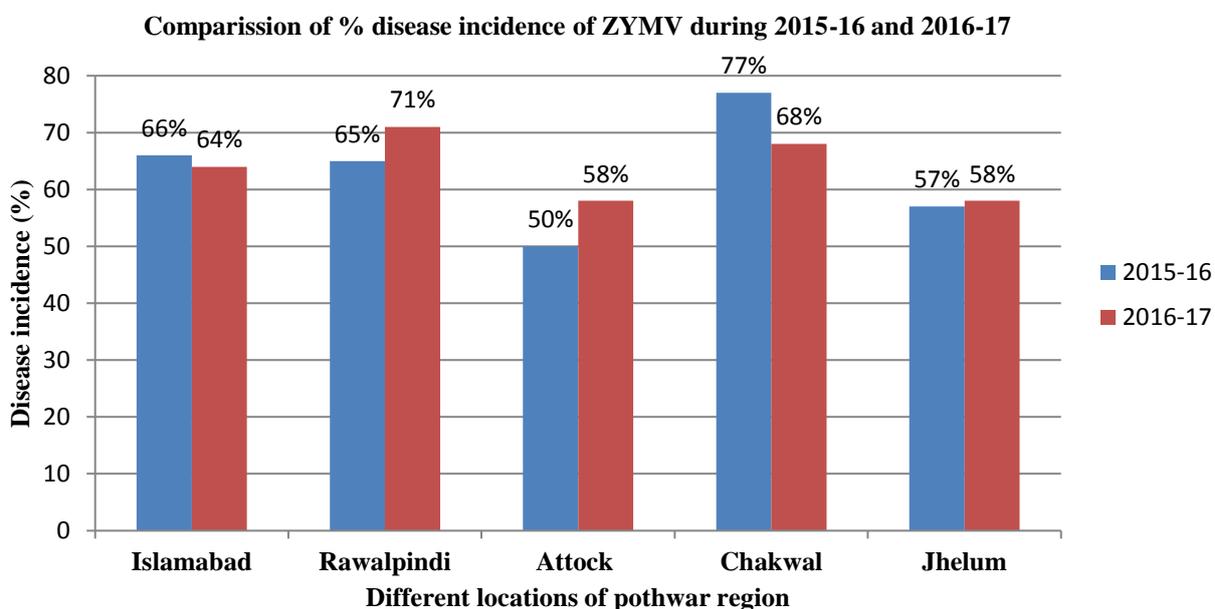


Figure 5. Comparison of disease incidence (%) of ZYMV in different areas of Pothwar region during 2015-16 and 206-17

Incidence of ZYMV in reaction plants and weed flora.

During survey different weed flora are also collected from cucumber fields which are subjected to DAS- ELISA to evaluate these weeds as alternate host of ZYMV. Along these weeds’ reaction plants are also established in isolated greenhouse

condition for symptoms and viral confirmation. During survey weed flora like (*Chenopodium* spp, *Portulaca oleracea*, *Cyprus rotundus*) were collected. Type of symptoms and ELISA results of weeds and indicator plants infected by ZYMV are shown in (Table 1).

Table 1. Prevalence of ZYMV in weeds and in tested plants

S. No.	Common Name	Scientific name	ELISA results	Symptoms
1	Chenopodium spp.	<i>Chenopodium amaranticolor</i>	++	CL, NL
2	Chenopodium spp.	<i>C. quinoa</i>	++	CL, NL
3	Tobacco	<i>Nicotiana tabacum</i>	+	LD
4	Cucumber	<i>Cucumis sativus</i>	+++	M, S,
5	Chili	<i>Capsicum annum</i>	-	#
6	Datura	<i>Datura stramonium</i>	+	S, ST
7	Luffa	<i>Luffa cylindrica</i>	+++	CL, M, LD
8	Tar	<i>Cucurbita moshata</i>	+	M, NL
9	Watermelon	<i>Cucumis melo</i>	-	#
10	Pea	<i>Pasium sativum</i>	+	CL, Lat
	Kulfa	<i>Portulaca oleracea</i>	+	S
	Deela	<i>Cyprus rotundus</i>	+	S

Keys for ELISA results: Strong (+++), Moderate (++), Mild (+), (-) No Reaction

Keys for symptoms: CL= Chlorotic lesion, NL= Necrotic lesion, (#) = No disease symptoms appear, M= Mosaic, ST= Stunting, S= Spots, LD = Leaf Distortion, Lat: Latent infection

Discussion

The survey conducted in all four districts of pothowar and capital territory shows that there is no single district free of infection of ZYMV. All the symptoms those are observed during survey as well as during greenhouse evaluation also reported by different scientist previously. [9, 16-18]. ZYMV present all over the world, Asia, Europe, Africa, Middle East, South as well as North America where cucurbits are grown and prevail in all environmental condition [7]. During 2015-16 highest disease was 77% recorded in Chakwal and during 2016-17 highest disease incidence was 71% recorded in Rawalpindi. Such type of findings is also observed by earlier scientists [19, 20]. 65 to 85% losses are also estimated by [19] in green house cultivated cucumber plants comparison of relative disease incidence of ZYMV during two consecutive years shows the increasing trend. i.e. 60.6% to 66%. As discus earlier that ZYMV prevail in wide environmental condition but the high temperature and vector populations help this virus to propagate more rapidly. Favorable environmental condition and high temperature also support the insect vectors. ZYMV symptoms develop at temperature 15-25°C but severe symptoms are observed

during temperature ranges from 25-40°C [21]. During survey Aphids colonies were observed on infected plant which were identified in Department of entomology, PMAS-UAAR. The identified aphids were *Aphis gossypii* and *Myzus persicae*. Aphid transmit ZYMV in non-persistent manner. In high disease incidence areas population rate of *Myzus persicae* was little higher then *Aphis gossypii*. Previous studies supported our observation that *Myzus persicae* transmit virus more efficiently then *Aphis gossypii*. [9, 10, 16, 22]. Weeds flora which are also collected during survey also gave positive results but the development of color during ELISA reaction was mild. Some other potential weeds (*Ranunculus sardous*, *Lamium amplexicaule*) were reported as reservoir of ZYMV [16, 20]). Symptoms development in mechanically inoculated plants shows that ZYMV have wide host range. These symptoms are also observed by scientist in earlier studies. In Tobacco [20] in Pea [23, 24] Cucumber, Chenopodium spp [7]. Development of such symptoms reduce the market value of fruit up to 95% [18] and in certain circumstances it destroys the whole crop. [25] ZYMV has highly devastating effect on cucurbits crop in this region so there is need to make strategies to control this

notorious virus. Weeding to eradicate the virus source in fields and control of aphid vector may remain helpful to reduce the dissemination of virus. As compare to other viruses like CMV, reservoir of ZYMV is very few so it is easy to control through weeding [26] Application of insecticide also reduce the population of aphid vector [27, 28]. Plastic mulches may also repel the aphid and may delay the spreading of virus [29, 30] but it may also affect the growth of plants. One of the most important and environment friendly method to control the virus is development of resistance cultivar and certified seed.

Conclusion

In conclusion present study indicate that *Zucchini yellow mosaic virus* (ZYMV) prevail in throughout the pothwar region of Pakistan. There is need to develop management strategies against such catastrophic pathogen. There is need to develop resistance cultivar of vegetables against this virus.

Authors' contributions

Conceived and designed the experiments: Z Asad, M Ashfaq, T Mukhtar & M Tariq, Performed the experiments: Z Asad, Analyzed the data: Z Asad, M Ashfaq, T Mukhtar & M Tariq, Contributed materials/analysis/ tools: M Ashfaq, Wrote the paper: Z Asad.

Acknowledgement

Author express his feeling for respectable supervisor Prof. Dr. Muhammad Ashfaq for his king supervision during whole research and also thankful to Co- Authors for sharing valuable time during manuscript write up and proof reading.

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