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# Serological and biological characterization of *Zucchini yellow mosaic virus* (ZYMV) infecting cucumber in Pothowar, Pakistan

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## Abstract

A survey was conducted during 2013-14 in open growing cucumber fields of Pothowar region in order to explore the prevalence of *Zucchini yellow mosaic virus* (ZYMV) through biological and serological assay. Leaves and fruits showing symptoms like mottling, mosaic, shoe string , knobby appearance, yellowing were collected randomly. Collected samples were tested byDAS-ELISA (Double Antibody Sandwich- Enzyme Linked Immunosorbant Assay) by using ZYMV virus specific antisera and through bioassay. Highest disease incidence was noted in Rawalpindi i.e. 59% followed by 53% in Islamabad, 33% in Attock as well as in Jhelum and 28% were recorded in chakwal. During this research study it is also monitored that aphid vector *Myzus persicae*, and *Aphis gossypii* transmit virus in non-persistent manner but the rate of transmission of *Myzus persicae* was little higher than *Aphis gossypii*.

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## Introduction

Cucumber (*Cucumis sativa* L.) is one of most important crop belongs to family Cucurbitaceae and for over 3,000 years cultivated by man (Denton and Adetula, 2003; Okonmah, 2011). Cucumber is delegate and succulent plant having high water proportion and large leaves covers whole fruit like canopy. Morphologically fruit is, elongated and cylindrical having tapered ends, mostly eaten as salads in unripe state and in tropical also used as stewed (Grubben, 1997). Due to economic importance, in Asia it has fourth positions after tomatoes, cabbage and onion (Remison and Eifediyi, 2011).And in Western Europe consider as second most valuable vegetable crop following tomato. (Phu, 1997).Cucumber is also a rich source of nutrients and it contain thiamine, vitamin C, niacin, phosphorous, iron, calcium, and have part of other healthful character. The low production is hampered by biotic and biotic factors and lack of resistant varieties. Among the biotic factor viral infectionis a standout amongst the most critical reasons of ailment.(Ozaslan *et al.*, 2006). Among these viruses, *zucchini yellow mosaic virus* (ZYMV) causes severe/ economic yield reduction in cucumber. Most prominent symptoms produce by ZYMV on leaf are mosaic, blistering, and size of leaf became reduce. Infected plants are stunted. Fruit symptoms encompass knobby areas which cause embossed deformation, and irregular skin coloring. (Desbiez and Lecoq, 1997).*Zucchini yellow mosaic* virus (ZYMV), genus Potyvirus belongs to family Potyviridae (Coutts *et al.* 2011) and was first reported from Italy in 1973 (Lisa *et al.*, 1981). Within a decade, the virus spread throughout the world, and become major threat to cucurbit crops (Desbiez and Lecoq, 1997). ZYMV is transmitted non-persistently by large number of aphid species (Katis *et al.,* 2006; Simmons*et al.,* 2013). Seed transmission occurs occasionally at very low rates in some cucurbit crops (Bananej *et al.,* 2008; Coutts *et al.*, 2011). According to Fletcher *et al.*(2000)ZYMV also transmit within cucurbit crops through contaminated field equipment. Wounds that were created during mechanical weeds eradication operations and certain vertebrates like rabbits enable ZYMV spread from plant-to-plant.(Riedle-Bauer.M*et al.,*2002).However, this idea is not supported by experimental evidence. Use of advance molecular techniques like application of nanotechnology, PDPR approaches and use of resistant varieties are consider as durable management strategies.In the present study, combination of visual symptom observations and enzyme-linked immunosorbent assay (ELISA) was used to evaluate the source of ZYMV from leaf tissue.

## Materials and methods

Sample collection

Cucumber field were visited in summer season 2013 and 2014 in pothwar region, viz Islamabad, Rawalpindi, Attock, chakwal and Jhelum where cucumber was grown. Leaf and fruit sample of cucumber exhibiting symptoms like yellow mosaic, necrosis, blister, distortion, fan-leaf appearance, shoe string, stunting were collected randomly. Further investigation bioassay and serological methods were carried out to confirm the viral nature of the disease and to identify the causative agent from collected samples. Bioassay and serological investigations were conducted.

*Bioassay*

Biological characterization and Pathogenicity test were held out by mechanical inoculation and through aphid transmission. For this purpose young leaves or tissues of fruit with characteristics symptoms were homogenized by 1/3 w/v in 0.05 M phosphate buffer having pH 7.2, containing 1% $Na_2SO_3$. (Ashfaq*et al.*, 2010). Following test plants were used to perform Bioassay test. i.e.*Chenopodiumamaranticolor, C. quinoa*, *Nicotianatabacum, Cucumis sativus cv, Capsicum annuum cv, Pasiumsativum, Daturastramonium, Luffacylindrica, Cucurbitamoshata, Cucumis melo*in control greenhouse condition. Development of symptom was investigated after every two days up to one month after inoculation (Lisa *et al.,* 1981; Lecoq *et al.*, 1981; Provvidenti*et al.*, 1984NatašaDukić*et al.*, 2002).

*Aphid transmission*

During survey intensive aphid colonies were observed

on diseased plants. These aphid species were collected and identified in department of Entomology- PMAS- Arid Agriculture University Rawalpindi. The Aphid species was *Myzuspersicae*, and *Aphis gossypii*.).During survey it is observe that population rate of *Myzuspersicae*was higher in Rawalpindi and Islamabad as compare to other localities. After identification colonies of aphid's was reared on healthy cucumber plant at 3-5 leaf stages in insect proof glass house attemperature (25±3°C) and provide a photoperiod of 8-10 hours. Aphid colonies were developed after 3 weeks,the aphids from reared colonies were picked up by gentle disturbance so that they withdraw their stylet through gentle breath and collected in a Petri dish with the help of moist brush. After starvation period of one-houraphids were transferred to infected plants and allowed for feeding for 2-3 minutes so that they would acquire the ZYMV virus. After acquisition feeding period of 2-3 mint aphids were transmitted on test plant in insect proof glass house for transmission feeding period of one hour. After transmission feeding period of one hour, aphids' vector was killed by spraying insecticide (Karate) @ 1% solution. The plants were observed every day for the development of symptoms. After 2 - 4 weeks of inoculation symptoms were noted, and ELISA was performed to confirm ZYMV's presence in the test plants.

*Serological assay*

Collected sample were subjected to DAS-ELISA (Double Antibody Sandwich- ELISA) as performed (Clark and Adam, 1977; Verma *et al.*, 2005) for investigation of virus from infected cucumber leaves collected from different localaties of pothowar region. Polystyrene plates were coated with antiZYMV antibodies (Bioreba AG, Switzerland), diluted 1:200 in coating buffer and incubated overnight at 4°C. Sap of infected leaves was extracted by using extraction buffer in mortar with pestle and double layered muslin cloth is used for sap filtration. Take 200µl of the filtered sap of each sample and then putinto 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). When the ELISA absorbance value was equal to two times higher than the average of absorbance value of the healthy tissue as well as negative control then samples were consider as positive for ZYMV infection. Commercial positive and negative controls (Bioreba) were included in ZYMV ELISA kit.

**Result and discussion**

Reaction of tested plant

Sample of infected cucumber crop collected from different localities of pothowar region during 2013 and 2014 showing virus like symptoms when inoculated on tested plant i.e*Chenopodiumamaranticolor, C. quinoa, Nicotianatabacum, Cucumis sativus cv, Capsicum annuum cv, Daturastramonium, Luffacylindrica, Cucurbitamoshata, Cucumis melo*shown symptoms describe in Table 1.

**Table 1.** Symptoms shown by tested plant after mechanical inoculation with ZYMV.

| Test plants | Symptoms |
|---|---|
| *Chenopodiumamaranticolor* | CL |
| *Chenopodium. quinoa* | NL |
| *Nicotianatabacum* | # |
| *Cucumis sativus* | M,LD,ST |
| *Capsicum annum* | # |
| *Daturastramonium* | S |
| *Luffacylindrical* | M,LD |
| *moshata* | M |
| *Cucumis melo* | LD,M |
| *Pasiumsativum* | Y |

Symptoms key:

CL= Chlorotic lesion, NL= Necrotic lesion, #= No disease symptoms appear, M= Mosaic, ST= Stunting, S= Spots, LD = Leaf Distortion, Y = yellowing.

**Table 2.** % Disease incidence and severity index of ZYMV infected sample.

| Location | No of sample +ve/tested | % Disease Incidence | Severity index |
|---|---|---|---|
| Islamabad | 28/53 | 53% | +++ |
| Rawalpindi | 25/40 | 59% | +++ |
| Attock | 14/47 | 33% | ++ |
| Chakwal | 10/35 | 28% | ++ |
| Jhelum | 13/40 | 33% | ++ |

These symptoms which are appeared on tested plant infected by ZYMV also describe by different researcher previously. The symptoms appear on, *Chenopodium*. *Quinoa, Cucumis melo, Chenopodiumamaranticolor, Cucumis sativus* plants during the investigation also describe by (Lesemann*et al*., 1983; Wang Y*et al*., 2000; Nataša Dukić *et al*., 2002; Jeffery, 2000 and; Müller *et al*., 2006) (Fig. 1). The symptoms appear on *Luffa cylindrical is also* supported by experimental investigation of Lisa *et al*., 1981; Lisa and Lecoq, 1984.

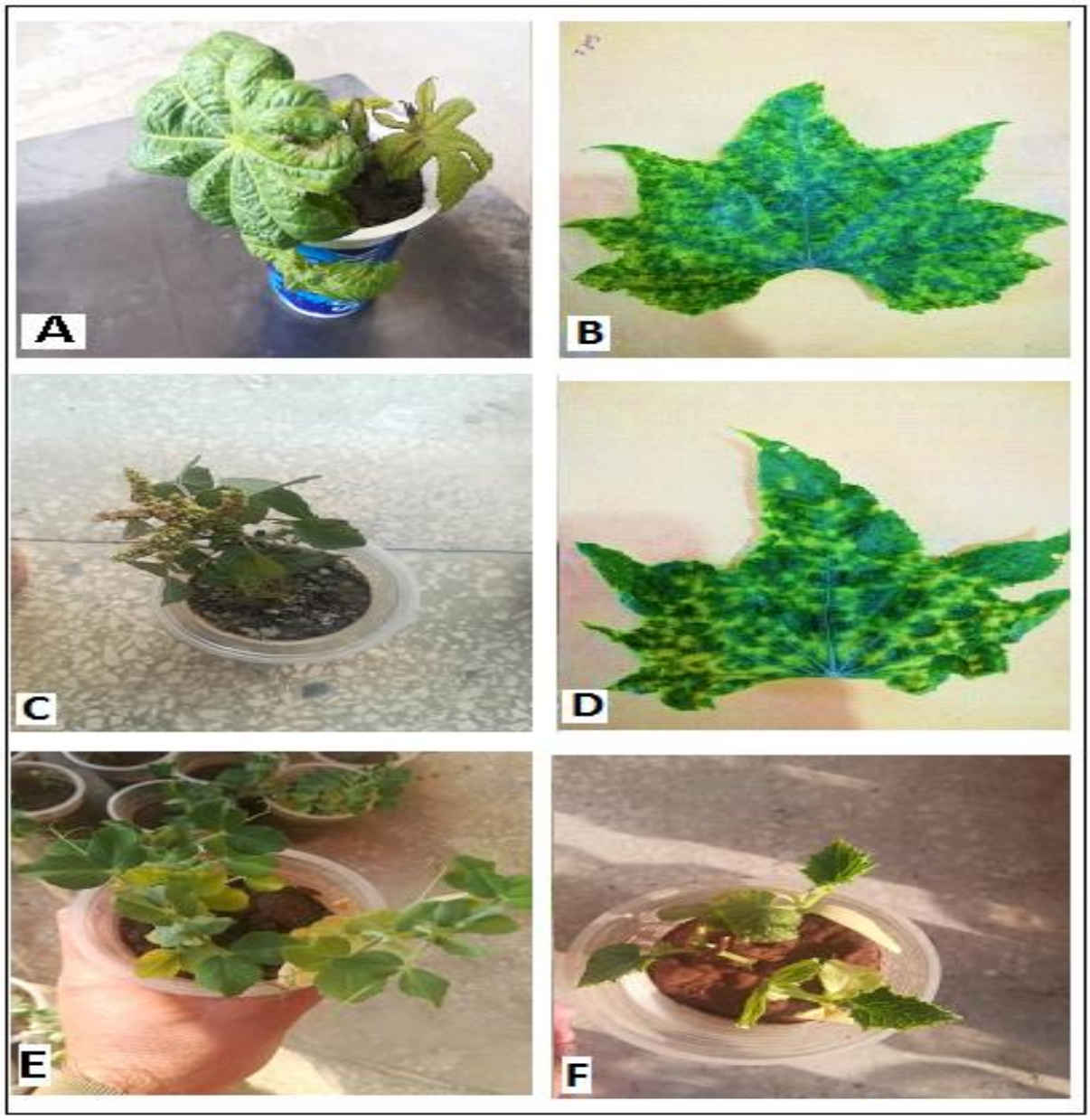


**Fig. 1.** Development of symptoms on reaction plants after mechnical inoculation with ZYMV.
(A) *Daturastramonium,* (B, D) *Luffa cylindrical* (C) *Chenopodiumspp* (E) *Pasiumsativum*(F) *Cucumis sativus*.

*Serological analysis*

Serological investigation confirmed that ZYMV is present in infected cucumber samples collected from different localities of pothowar region as well as mechanically inoculated plant samples but severity of ZYMV infection to cucumber crop varies according to location. Severity of ZYMV infected cucumber sample, which are tested through DAS-ELISAfrom each locality) are shown in Table 2.

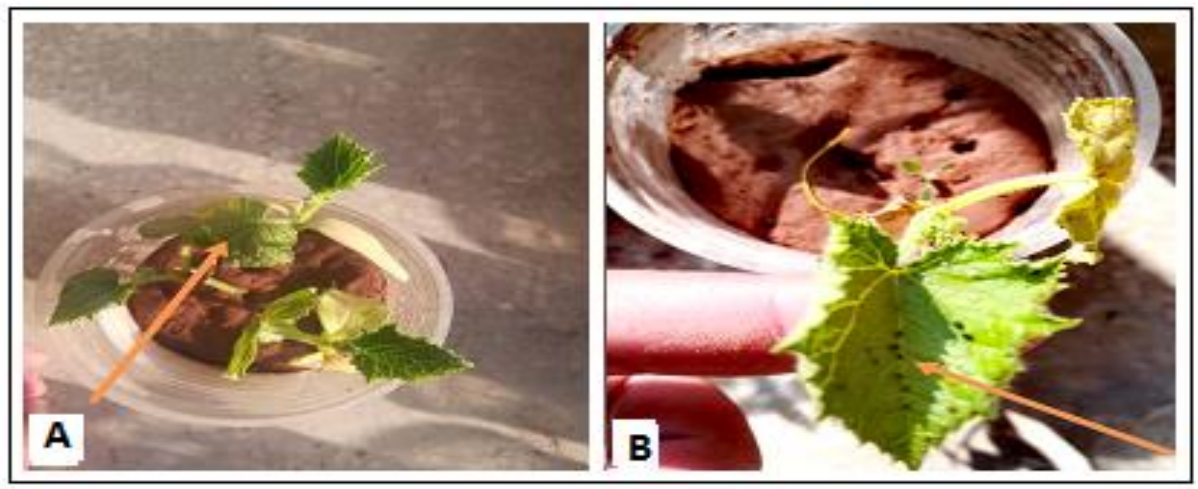


**Fig. 2.** Virus transmission through Aphid vector (A) Development of symptoms on healthy Cucumber plant after virus transmission through aphids (B) Aphids feeding on infected plant.

Severity may attribute to presence of different vector species or it may indicate the presence of different strain of virus. Serological diagnosis of viruses is suitable and easy to handle. DAS- ELISA is use worldwide for identification of plant viruses (Yuki *et al.*,2000).

*Aphid transmission*

Two types of aphid species are used to investigate the transmission of ZYMV. One was*Myzuspersicae* and other was *Aphis gossypii*. These two species are identified during survey. During the experimental trail it is observe that both aphid species transmit the ZYMV but rate of transmission was different.(Dombrovsky*et al.*, 2005) Transmission rate of *Myzuspersicae*. Was little faster as compare to*Aphis gossypii* (Martínez M C D *et al.,* 2004) *Invivo* and *invitro* efficient transmission of ZYMV and WMV through *M. persicae*as compare to *Aphis gossypii* was also reported byCastle *et al.,* 1992.Transmission of virus is also conformed through reinoculation (Nataša Dukić*et al.*, 2002). It is also observed thatAphid infested plants show the symptoms similar to those which are observe in fields during survey (Fig.2).Katis*et al.,* 2006 also repoted that ZYMV is transmitted through different aphid species.

**Conclusion**

In pothowar region during survey (2013-14) it is observed that cucumber plants depict different symptoms like mosaic, leaf deformation, knobby outgrowth on fruits,leaf yellowing, chlorotic as well as necrotic spots. Etiological analysis shows that these symptoms are attributed to plant pathogenic viruses. Serological diagnosis proves that causal agent of these symptoms is ZYMV, most destructive potyvirus in this region. Aphid colonies are also observed during survey which are identified i.e*Aphis gossypii, Myzuspersicae,* and tested .Aphid transmission test results indicate that this virus is transmitted through apids in non-persistent manner but rate of transmission is little bit vary.

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