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

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**Key words:** ZYMV, Prevalence, DAS-ELISA, Incidence, Cucumber.

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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 by DAS-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 the most important crops belonging to the family Cucurbitaceae and for over 3,000 years cultivated by man (Denton and Adetula, 2003; Okonmah, 2011). Cucumber is a delegate and succulent plant having high water proportion and large leaves covering the whole fruit like canopy. Morphologically, the fruit is elongated and cylindrical having tapered ends, mostly eaten as salads in the 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 contains 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 infection is a standout amongst the most critical reasons of ailments. (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 (Banan 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 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 the 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$_2$SO$_3$. (Ashfaq et al., 2010). Following test plants were used to perform Bioassay test. *i.e. Chenopodium amaranticolor, C. quinoa, Nicotiana tabacum, Cucumis sativus cv, Capsicum annuum cv, Psium sativum, Datura stramonium, Luffa cylindrica, Cucurbitamoshata, Cucumis meloin* 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., 1984 Nataša Dukić 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 Myzus persicae, and Aphis gossypii). During survey it is observe that population rate of Myzus persicae 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 at temperature (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-hour aphids 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 localities 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 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). 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 Chenopodium amaranticolor, C. quinoa, Nicotianatabacum, Cucumis sativus cv, Capsicum annuum cv, Daturastramonium, Luffacylindrica, Cucurbitamoshata, Cucumis melo shown symptoms described in Table 1.

<table>
<thead>
<tr>
<th>Test plants</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chenopodium amaranticolor</td>
<td>CL</td>
</tr>
<tr>
<td>Chenopodium, quinoa</td>
<td>NL</td>
</tr>
<tr>
<td>Nicotianatabacum</td>
<td>#</td>
</tr>
<tr>
<td>Cucumis sativus</td>
<td>M, LD, ST</td>
</tr>
<tr>
<td>Capsicum annuum</td>
<td>#</td>
</tr>
<tr>
<td>Daturastramonium</td>
<td>S</td>
</tr>
<tr>
<td>Luffacylindrica</td>
<td>M</td>
</tr>
<tr>
<td>moshata</td>
<td>M</td>
</tr>
<tr>
<td>Cucumis melo</td>
<td>LD, M</td>
</tr>
<tr>
<td>Psidium sativum</td>
<td>Y</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
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<th>Test plants</th>
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<tr>
<td>Chenopodium amaranticolor</td>
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Table 2. % Disease incidence and severity index of ZYMV infected sample.

<table>
<thead>
<tr>
<th>Location</th>
<th>No of sample +ve/tested</th>
<th>% Disease Incidence</th>
<th>Severity index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Islamabad</td>
<td>28/53</td>
<td>53%</td>
<td>+++</td>
</tr>
<tr>
<td>Rawalpindi</td>
<td>25/40</td>
<td>59%</td>
<td>+++</td>
</tr>
<tr>
<td>Attock</td>
<td>14/47</td>
<td>33%</td>
<td>++</td>
</tr>
<tr>
<td>Chakwal</td>
<td>10/35</td>
<td>28%</td>
<td>++</td>
</tr>
<tr>
<td>Jhelum</td>
<td>13/40</td>
<td>33%</td>
<td>++</td>
</tr>
</tbody>
</table>

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 (Lesemannet al., 1983; Wang Yet 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 mechanical 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-ELISA from 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 *Myzus persicae* and other was *Aphis gossypii*. These two species are identified during survey. During the experimental trial it is observe that both aphid species transmit the ZYMV but rate of transmission was different. (Dombrovskyy et al., 2005) Transmission rate of *Myzus persicae*. Was little faster as compare to *Aphis gossypii* (Martínez M C D et al., 2004) In vivo and invitro efficient transmission of ZYMV and WMV through *M. persicaeas* 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 that Aphid infested plants show the symptoms similar to those which are observe in fields during survey (Fig.2). Katiset et al., 2006 also reproted 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*, *Myzus persicae*, 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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References


https://doi.org/10.1007/s00705-011-1102-0


https://doi.org/10.1080/01140671.2000.9514118


that causes zucchini yellow mosaic. Phytopathology 71, 667-672.


