INCIDENCE OF APHID BORNE VIRUSES AND THEIR VECTOR DYNAMICS INFECTING POTATO CROP IN DIFFERENT DISTRICTS OF AZAD JAMMU AND KASHMIR, PAKISTAN

Irfan Tahir¹, Zohaib Asad², Muhammad Usman Shahid², Tahira Shafique³, Saiqa Shafique⁴, Nasir Uddin⁵, Rameesha Abid⁶, Anis Safir⁷, Hafiza Sehrish kinai⁸, Waqas Safir⁹*

¹Department of Plant Pathology, University of Agriculture Peshawar, Pakistan.
²Department of Plant Pathology, PMAS, Arid Agriculture University Rawalpindi, Pakistan.
³Department of Biochemistry, University of Agriculture Faisalabad, Pakistan.
⁴Department of Microbiology, Government College University Faisalabad, Pakistan.
⁵Directorate of Agricultural Research System, Peshawar 25120, KPK, Pakistan.
⁶Department of Biotechnology, University of Sialkot, Sialkot 51310, Pakistan.
⁷Department of Environmental Sciences, University of Peshawar, KPK, Pakistan.
⁸Department of Microbiology, Quaid-i- Azam University islamabad, 44000, Pakistan.
⁹Xinjiang Key Laboratory of Biological Resources and Genetic Engineering, College of Life Science and Technology, Xinjiang University, 830046, Urumqi, Xinjiang, China.

Corresponding Author e-mail: waqaskustodian@gmail.com

Abstract

Potato crop production is hampered by several fungal, viral, and bacterial diseases. Among the viral *Potato leaf roll virus* (PLRV), *Potato virus Y* (PVY), *Potato-mop-top-virus* (PMTV), *Potato virus S* (PVS) and *Potato virus X* (PVX) are the most important. Potato growing fields were surveyed during 2016 and 2017 to determine the incidence of two aphid-borne viruses, PVY and PLRV and to explore the dynamics of potato aphids in four potato-producing district of Azad Jammu & Kashmir (AJK), Pakistan. During survey 930 leaf samples were collected from various locations of Bagh, Sudhnoti, Poonch and Neelum. Parameters determined included aphid population, symptomology, DAC-ELISA based virus incidence, rate of co-infection and disease severity. Overall relative disease incidence of PLRV and PVY was 48.71% and 53.23% respectively. The highest average aphid’s population per field was 390.27 in Sudhnoti due to the low altitude of the surveyed locations. Three aphid species *Myzus persicae, Myzus ornatus*, and...
Macrosiphum euphobiae were identified in AJK potato fields. The maximum co-infection was recorded from Poonch. It was found that aphid populations decreased with the increase of altitude. The incidence of PLRV and PVY were high where aphid population was high. Therefore, it is necessary to promote awareness among the farmers on virus and aphid symptoms identification and to recommend use of healthy, uncontaminated certified potato seed.

**Keywords:** Aphid; Potato virus Y; Potato leaf roll virus; viral incidence; DAC-ELISA

1. **INTRODUCTION**

Potato (Solanum tuberosum L) is categorized as tuber crop that is classified in family Solanaceae. Nutritionally, the potato tuber contains starch (18%), protein (2%), water (70%) and low amount of vitamins, minerals and trace elements (1%) (Nadeem et al., 2011). Potato grown in more than 140 countries (Haase, 2007). Potato is one of most the valuable vegetable crop of Pakistan, with 176.2 thousand hectares and an annual production approximately 4,134.6. In Azad Jammu and Kashmir (AJ&K) potato is a fourth crop after rice, maize, and wheat (Jamro, Tunio, Buriro, & Chachar, 2015). Potato crop plays a vital role in the overall economy of poor families being considered as cash crop of poor families of AJ&K and Gilgit-Baltistan. Additionally, it has a significant impact on how poor families' socioeconomic situation develops (Alam, Kobayashi, Matsumura, Ishida, & Mohamed, 2012). Potatoes can be grown up to 4000 meters above sea level and up to 40° north latitudes. Potato is considered as a cool season crop. Approximately 500-700mm rainfall is favorable for maximum potato production. Minimum temperature for potato production is 16-19 °C and 21°C as optimum (Van der Zaag, 1987). The production of the potato is impeded by various bacterial, viral, and fungal diseases. Among the viral pathogens Potato Virus Y (PVY), Potato virus S (PVS), Potato-mop-top-virus (PMTV), Potato leaf roll virus (PLRV) and Potato Virus X (PVX) has significant importance (Mughal, Khalid, & Hussain, 1986). In Pakistan PLRV, PVX and PVY damage the crop at large scale(Abbas et al., 2012). PLRV and PVY cause more catastrophic effect because they are transmitted through seed tuber and through aphid. Lack of natural host resistance and use of unhealthy potato tubers may aggravate the prevalence of these viruses(Mughal et al., 1986). PLRV (family Lutoviridae's genus Polerovirus) comprises of single stranded, positive sense, monopartite, and linear approximately 6 kb long RNA genome (Talianksy, Mayo, & Barker, 2003). It is transmitted
by aphids and primary symptoms are restricted to phloem bundles. Symptoms of infection on infected plants include rolling of upper and lower leaves, ridding and yellowing of leaves, dwarfing, stunting (reduces tuber weight and size) in potato crop (Gibson, Pehu, Woods, & Jones, 1990). Many types of aphid species are reported vector of PLRV in potato crop. *Myzus persicae*, one of the most effective aphid vectors, transmits the PLRV in a persistent, circulating, and non-propagative way. PLRV prevalence is sharply rising throughout Pakistan's potato-growing regions (Mughal, 2003) and it causes losses of up to 70% (Ahmed & Ahmed, 1995).

*Potato virus Y* (Genus *Potyvirus*; family *Potyviridae*) (Dougherty & Carrington, 1988) transmitted by *Myzus persicae* in non-persistent way (Sigvald, 1984). Numerous symptoms are produced by this virus, including severe mosaic, mottling, necrosis in the vein on the underside of the leaflets, and stipple streak (Barker & Harrison, 1984). According to (Khalid, Iftikhar, Munir, & Ahmad, 2000) PVY causes 80% losses in Pakistan. At present there are no reports regarding the prevalence of these aphid-borne viruses infecting potato in AJ and K. Keeping in view the importance of these viruses, this study was design to estimate the occurrence of aphid borne viruses. PVY and PLRV in potato explore the dynamics of potato aphids in four potato producing districts of Azad Jammu & Kashmir, Pakistan.

### 2. MATERIALS AND METHODS

#### 2.1. Aphid surveillance and leaf sampling

Visual assessments of viral incidence and field surveys for aphids on potato leaves were carried out in four districts (Bagh, Poonch, Sudhnuti and Neelum) in the Azad Jammu and Kashmir (AJk) region, during two consecutive years (2016 and 2017). From four districts different locations were selected for leaf sampling and aphid population dynamics (Table1). From each location two farmer fields were randomly selected for sample collection, to estimate the visual virus incidence and to analyze the population dynamics of vectors. For leaf samples collection, fields were divided in to three third, 100 plants were randomly selected and 50 samples were collected from these plants by using “W” sampling method as described by (Teulon, Eastop, Stufkens, & Harcourt, 1999). Elevations of sampling sites were also measured. Mean disease visual incidence was calculated on the basis of symptoms produced by aphid-borne viruses (PLRV and PVY) and disease severity was also estimated
as defined by (Khan, Ullah, & Iqbal, 2006) and (Mughal & Khan, 2001). Percent disease incidence was computed by following equation (Srivastava, Khalid, Singh, & Sharma, 2010).

\[
\text{Estimated \% disease Incidence} = \frac{\text{No. of infected plants}}{\text{Total plants}} \times 100
\]

2.2. Estimation of aphid population dynamics and viral incidence

Disease incidence in the farmer fields were recorded on the basis of typical symptoms produced by aphid-borne potato viruses such as PLRV. It was identified based on characteristics symptoms such as leaf rolling chlorosis in upper leaves, while rugoses (mosaic, leaf necrosis and stunting) was a major symptom of PVY in the fields. To investigate the dynamics of aphid population 15 plants were randomly selected. Aphids were collected from three mature compound leaves (top, middle and bottom) from each selected plant. Aphid collection was done by clipped the leaves and brushed off gently with the help of camel hair brush in a Petri-plates. A universal bottle half-filled with 70% ethylc alcohol was used to transfer the collected aphids for later study (Karasev et al., 2010). Identification of these aphids was performed under stereo zoom microscope (Zoom NTB-3A). Following a taxonomic key (Teulon et al., 1999).

2.3. Serological analysis

Serological analysis was performed by using Direct Antigen Coating–Enzyme Linked Immunosorbsent Assay (DAC- ELISA) as elaborated by (Howell, 1977) and (Bashir, Ahmad, & Ghafoor, 2002). Extraction buffer, (pH 7.4) was used to ground potato samples followed by coating of micro plates well (200 µl/well) with antigen and overnight incubation was done at 4°C. After that, the micro plates were cleaned three times with PBS that included 0.05 percent Tween-20. Cross-absorbed crude antiserum (PLRV and PVY) was added into the plate wells and incubate once more at 4°C for an overnight period using a dilution of 1:500 in the coating buffer (pH 9.6) having antibody. Diluted (1:1000) goat-anti-rabbit conjugate in conjugate buffer (pH 7.4) was filled into wells followed overnight incubation at 4°C. After incubation, nitro phenyl phosphate substrate was filled into wells placed at room temperature in dark, condition. Eventually, at room temperature in the dark, the plates were tightly sealed with aluminum foil and incubated for 60 min. The changes in color were observed visually and photoelectrically with the ELISA Reader at 405 nm. If a sample's values were greater
than three standard deviations from the average of the negative controls or equal to or close to those of the positive controls, it was considered to be infected. The ELISA kit that is available commercially was used to get the positive and negative controls (Bioreba AG, Switzerland).

2.4. Statistical analysis

To determine the impact of viruses on the potato crop, the collected data was analyzed using statistics 8.1 (analytical software) (Steel, 1997). The aphids were calculated, and information was analyzed using standard statistical tools (8.1) to calculate the average number of aphids per leaf. The species of each individual aphid was counted, and the results were then analyzed by using appropriate statistical tool to calculate the average number of aphid species per leaf.

2. RESULTS

3.1. Estimation of Aphid population dynamics

The vectors of both viruses were recorded in all studied locations. Three aphid species *Myzus persicae*, *Myzus ornatus* and *Macrosiphum euphorbiae* were identified during survey. The average maximum aphid population was recorded in Sudhnoti (390.27), followed by Poonch (320.44) and Bagh (310.40) as shown in Table 1. The least aphid population was recorded in Neelum (246.83). (Table 3). Regression analysis shows that aphid population decreased when altitude increase, with the increased in 1000 meter of altitude the number of vector population decrease by 35.8(Δ=-0.0358). The linear model described 35.8 variability of change in mean aphid numbers (R² = 0.7505) (Figure 1).
Correlation analysis shows positive relationship between incidence of PLRV and aphid population. There is direct relationship between aphid population and PLRV incidence ($R^2 = 0.7505$) (Figure 2). Similarly, correlation analysis of aphid population and PVY exhibits positive relation. When aphid population increased incidence of PVY also increased ($R^2 = 0.6505$). Correlation data proved that aphid population is directly proportion to virus incidence (Figure 3).

Figure 2. Relationship between aphid incidence of PLRV.

Figure 3. Relationship between aphid incidence of PVY.
Table 1. Association of aphid population with altitude and disease incidence in Azad Jammu and Kashmir.

<table>
<thead>
<tr>
<th>Districts</th>
<th>Location</th>
<th>Altitude(meter)</th>
<th>Aphid population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bagh</td>
<td>Sudhangali</td>
<td>2133</td>
<td>320.21</td>
</tr>
<tr>
<td></td>
<td>Dothan Thub</td>
<td>2499</td>
<td>300.60</td>
</tr>
<tr>
<td></td>
<td>Rawalakot</td>
<td>1637</td>
<td>360.44</td>
</tr>
<tr>
<td>Poonch</td>
<td>Banjosa</td>
<td>1950</td>
<td>280.34</td>
</tr>
<tr>
<td></td>
<td>Thrarkhel. I</td>
<td>1371</td>
<td>380.54</td>
</tr>
<tr>
<td>Sudhnoti</td>
<td>Thrarkhel. II</td>
<td>1371</td>
<td>400.00</td>
</tr>
<tr>
<td>Neelam</td>
<td>Sharda</td>
<td>1980</td>
<td>283.44</td>
</tr>
<tr>
<td></td>
<td>Arang kel</td>
<td>2553</td>
<td>246.83</td>
</tr>
</tbody>
</table>
3.2. Disease incidence

Disease incidence results shows that during 2016 in case of PLRV highest disease incidence was observed in districts Sudhnoti (58.13%) while lowest was observed in district Neelum (40%). During 2016 highest PLRV incidence was also observed in district Sudhnoti i.e. 55.11% while least disease incidence was observed in District Poonch i.e., 42.06%. During 2017 highly infested district was district Bagh had disease incidence of 62.66% in case of PVY as shown in Figure 4,5 while lowest was observed in districts Sudhnoti i.e., 53.45%. In case of PLRV sudhnoti was highly infested district had disease incidence of 52.44% while lowest was observed in District Neelum i.e. 42% as shown in Figure 4, 5. Overall relative disease incidence of PLRV and PVY was 48.71% and 53.23% respectively as shown in Figure 6. ELISA results show that not even single site was free from infection.

![Figure 4. Disease Incidence of PVY and PLRV in 2016 and 2017 at district level](image-url)
Figure 5. Disease incidence of PLRV and PVY at different location of AJK.

Figure 6. Overall relative incidence of PLRV and PVY during 2016 and 2017

3.3. Disease severity and mixed infection
Disease severity and mix infection was also estimated during two consecutive years 2016-17. Disease severity of PLRV and PVY was gauged by following disease rating scale suggested by Khan et al., (2006) and Mughal and Khan, (2001) respectively. According to disease rating scale for PLRV (0-5) the highest disease severity was recorded in Sudhnoti (1.36) and least severity index was recorded in Neelum. According to disease rating scale (0-4) for PVY the highest severity in 2017 was recorded in Sudhnoti (1.56) foPoonch (1.48) and least in Neelum (Figure 7).
3. DISCUSSION

In the current study, four potato-growing districts in Azad Jammu and Kashmir were examined for the presence and distribution of aphid-borne potato viruses and their vector. The present study indicated that not even single location was free from aphid-borne viruses (PLRV and PVY). During two consecutive years 2016-17 relative percent incidence of viruses were estimated in selected locations of AJK. Overall relative disease incidence of PLRV and PVY was 48.71% and 53.23% respectively (Mughal & Khan, 2001). The occurrence of PLRV that (Arif, Mughal, Khalid, & Hassan, 1995) previously reported is lower than that of the recent report. The maximum percentage of PLRV was found in plain areas at 44 percent, 53 percent from non-registered seed, and 11 percent from registered seed (Arif et al., 1995). The incidence of PVY in earlier research is lower than in the current report. Pakistan's potato crop had the greatest PVY disease incidence, with rates ranging from 5 to 25 percent (Mughal & Khalid, 1985). The decreased incidence of PLRV compared to PVY is due to the easier detection of PLRV symptoms and the farmer's removal of infected plants prior to the survey, or it may be due to M. persicae, the most effective PLRV vector, being less prevalent than aphid that transmit PVY. The other factor that may favor the high
incidence of these viruses such as consecutive use of potato tubers, intensive presence of aphid vector, uncertified virus infected seed, susceptibility of plant against potato viruses, low altitude and presence of aphid vectors, insufficient knowledge about viral infections among potato growers, poor agronomic practices and favorable climatic condition for virus ecology that participate to high prevalence of these viruses in these areas.

Average maximum percentage of mixed infection was recorded in Sudhnoti (27.52% and 30.33%) in 2016 and 2017 respectively and minimum was observed in Neelum in consecutive potato growing season. Rise in disease severity and an accumulation of viral particles are linked to the mixed infection. It causes severe losses. Our results are in line with those of who claimed that synergistic infection of PLRV and PVY causes substantial losses that diminish tuber size and results in very poor crop quality as compared to infection caused by both viruses separately (Wang et al., 2011). The estimation of aphid’s population was observed under natural climatic conditions during survey period. The average dynamics in aphid population was reported in four districts of AJK. The maximum and minimum aphid population was reported from Sudhnoti (390.27) and Neelam (246.83). The biodiversity of aphid population depends on altitude, climatic conditions of the region specially direction of wind, rainfall, temperature and humidity. Data indicated that aphid population fluctuates with altitude when altitude increase, the aphid population decreased. The areas, where are aphid population lower, suitable for potato seed production. Our findings are similar with those work of (Capinera, 2001) and (Thomas, 2002) who reported that where aphid number is less than 3-10/100 leaves are suitable for potato production and multiplication. Climatic conditions affect the biodiversity of aphid population. Climatic condition (temperature, humidity and air fall) also effects the aphid populations. The ideal temperature range for aphid activity is between 22°C and 26°C; if the temperature exceeds 26°C, the activity of flight is reduced (Taylor, 1963). Our results are in lines with (Were et al., 2013) who find that aphid population decreased at high altitude. We observe that temperature and rainfall also effect the aphid population and disease incidence similar observation are also noticed by (Kuroli, 1999). During study survey it is noticed that aphid population was highest when climatic conditions were dry and warm. Similar observation was also mentioned in previous work (Aheer, Munir, & Ali, 2007; Lamb, Wise, & MacKay, 1997). Three aphid’s species *Myzus persicae*, *Macrosiphum euphorbiae* and *Myzus ornatus* were identified during survey which transmits the virus. Our finding regarding vector prevalence in potato fields are also strengthen by the finding of (Muthomi, Kinyungu, Nderitu, & Olubayo, 2011). During
surveys it was observed that there is positive relationship among aphid population and altitude. As altitude rises, the aphid population declines (Were et al., 2013). This results shows that PLRV and PVY and their vector are prevalent in AJK and Pakistan. Comprehensive surveys of potato fields must be conducted in all districts of AJK to explore other potato viruses at molecular basis. Production of virus-free and certified seed is need of hour for successful production of potato crop in AJK.

4. Conclusions

It is concluded both aphid borne viruses are prevailing in throughout the surveyed districts of AJK. There is need to identified these pathogens at molecular basis and also to develop management strategies against such catastrophic viruses.

**Funding:** This research received no external funding.

**Acknowledgments:** We are grateful to the staff and researchers at "The Agricultural University, Peshawar" for helping us in this work.

**REFERENCES**


