



## Research Article

# Exploring Incidence of Potato Viruses in Major Growing Areas of Potato in Khyber Pakhtunkhwa, Pakistan

Zafrullah Khan<sup>1</sup>, Shah Alam Khan<sup>1</sup>, Tamana Bakht<sup>2</sup>, Luqman<sup>5\*</sup>, Noor Muhammad<sup>1</sup>, Naeem Khan<sup>3</sup>, Iqbal Munir<sup>4</sup>, Ruqayya Qazi<sup>1</sup> and Shazia<sup>2</sup>

<sup>1</sup>Department of Plant Protection, The University of Agriculture, Peshawar, Khyber Pakhtunkhwa, Pakistan; <sup>2</sup>Department of Environmental Science, Department of Agriculture, Department of Forestry Shaheed Benazir Bhutto University, Sheringal Dir Upper, Pakistan; <sup>3</sup>Department of Weed Science and Botany, The University of Agriculture, Peshawar, Khyber Pakhtunkhwa, Pakistan; <sup>4</sup>Institute of Biogenetics and Engineering, The University of Agriculture, Peshawar, Khyber Pakhtunkhwa, Pakistan; <sup>5</sup>Department of Agriculture Extension, Khyber Pakhtunkhwa, Pakistan.

**Abstract** | The Enzyme Linked Immunosorbant Assay (ELISA) test of potato leaves collected from different areas within three (3) selected divisions in Khyber Pakhtunkhwa, Pakistan was carried out in 2016. The region-wise results showed the over all incidence of potato virus Y (PVY) to be highest in all the three divisions on potato crop followed by the incidence of potato leaf roll virus (PLRV). The PVY and PLRV incidence in Malakand division was noted by 62.7 and 44.6 %, respectively. In Peshawar division the incidence of PVY was 55.4% and that of the PLRV was 40.7% and the incidence of PVY and PLRV was 50.7 and 37.3%, respectively in Hazara. The field-wise results of Malakand region showed the highest incidence of PVY and PLRV in Kalam was found by 70 and 50% followed by Miandam (62 and 46 %) and the lowest incidence was recorded in Malamjaba (56 and 38%). In Peshawar the highest incidence of PVY and PLRV was noted on potato crop in Warmarh (66 and 46%) followed by Regi (52 and 32%) and the lowest was noted in Pul Dheri Mehra (48 and 44%). In the Hazara potato crop in Kaghan had 62 and 54% PVY and PLRV incidence followed by Bagnotar (48 and 36%) and the lowest incidence was noted in Kalanderabad (42 and 22%), respectively. Results obtained by this study indicating that both PVY and PLRV are major viruses of potato crop in selected potato growing areas of KPK province which could pose serious threat to the potato crop, development of resistance cultivar against vector insect could be recommended.

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**\*Correspondence** | Luqman, Department of Agriculture Extension, Khyber Pakhtunkhwa, Pakistan; **Email:** luqmanaup@yahoo.com

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## Introduction

Potato (*Solanum tuberosum* L.) being an important vegetable tuber crop in Pakistan can be attacked

by various viral diseases such as the potato virus X (PVX), potato mop top virus (PMTV), potato virus S (PVS), potato virus A (PVA), alfalfa mosaic virus (AMV) and potato virus M (PVM) *Myzus persicae*

being dual insect pest not only harm the potato crop by direct feeding but also play major role as vector of many viruses which can cause different viral diseases in potato (Valkonen, 2007). The most harmful viruses of potato in Pakistan are reported to be the potato virus Y (PVY), potato leaf roll virus (PLRV), (Qamar *et al.*, 2015). The viral infection can be from no-to-100% in potato plants. Among the different vectors of disease transmission into the potato plants (e.g. potato psyllid (*Bactericera cockerelli*, Sulc)) *M. persicae* can also make major transmission of disease into the crop (Yao *et al.*, 2016). Among the different viruses, the PVY and PLRV are the two significant viruses of potato crop in Pakistan (Mughal *et al.*, 1988). These two viruses are widely spread and are major threats to the potato crop in Pakistan (Hameed *et al.*, 2014). Virus infection in potato crop cause major reduction in the size and yield of potato tubers. Together viruses can cause up to 83 % yield losses to the potato crop (Mughal and Khalid, 1985) and c. 50 to 90 % yield losses can be caused only by the infection of PVY and PLRV (Qamar *et al.*, 2015). The PVY of *Potyvirus* family and *Potyvirus* genus) and the PLRV of *Luteoviridae* family *polerovirus* genus can both act as vectors of many important plant viruses.

The typical population of *M. persicae* on potato plant is c. 320 *M. persicae* per plant in Khyber Pakhtunkhwa. Such a large number of *M. persicae* on potato plants can be expected to transmit significant number of viruses such as the PVY and PLRV into the crop. The vectors can transmit the PVY and PLRV in a non-persistent and persistent way, respectively (Radcliffe and Ragsdale, 2002; Boquel *et al.*, 2011). A good number of potato cultivars e.g. Sahiwal Red, Sarpo Mira and Rocco all have shown resistance to the *M. persicae* infestation (see tables of Antixenosis and Antibiosis in Resistance Chapter). The resistance potato cultivars may also play a role in reducing the incidence of viral diseases in potato crops growing areas. The incidence and distribution of virus infection could be varied on different potato cultivars in the field conditions. Thus it may be important to explore the incidence and distribution of the two widely spread viruses (PVY and PLRV) in potato cultivated fields in Khyber Pakhtunkhwa, Pakistan.

Visual symptomatic detection and confirmation of viral diseases in potato crop have been practiced since long time ago, however, visual detection of symptoms of viral infection in potato crop cannot

be scientific and accurate in all cases. For example, in case of some strains of PVY in latent infection, plants do not indicate disease symptoms to detect and recognize the viral infection well on time and easily. Other conventional tests such as the neutralization, complement fixation and indirect immunofluorescence are all reliable however they possess a number of limitations. For example their practical complication, extensive hands on time, titration of reagents and specimens dilution and some times inaccurate-positive or negative results. Serological test of detection and measurement of viral incidence in potato crop is a scientific, a more reliable and more accurate method. This method has been used widely by the scientific community around the world (Singh *et al.*, 2017). The double antibodies sandwich enzyme linked immunosorbent assay DAS-ELISA. In this present study serological method was used to explore the incidence of PVY and PLRV viruses in three major potato growing regions (Malakand, Peshawar and Hazara) of Khyber Pakhtunkhwa. This present study assessed the incidence and distribution of two major viruses of potato crop (PVY and PLRV) in three major potato growing areas in Khyber Pakhtunkhwa province of Pakistan in 2016. The main objective of this present study was to detect the incidence of PVY and PLRV viruses in potato fields in three major growing areas in Khyber Pakhtunkhwa, Pakistan and to record if there was resistance of potato cultivars to the viruses under field condition.

## Materials and Methods

### *Field surveys and samples collection*

Surveys of potato fields in three different regions i.e., Peshawar, Malakand and Hazara divisions of Khyber Pakhtunkhwa province of Pakistan were conducted in 2016. The surveys of Malakand and Hazara divisions were undertaken during August 2016 and survey of Peshawar division was undertaken during November 2016. The three different regions for this study were selected on the basis of their major potato cultivation status. In each of the three divisions, three typical farmer fields under potato cultivation were selected. In each selected potato field 50 samples of potato leaves (150 samples per region) were randomly collected from plants of potatoes at c. 6 to 8 weeks growth stage. Potato samples showing paleness and upward rolling of young leaves, stunted growth and yellowing of leaf margins were detected serologically against PLRV. To make random collection of leaf samples from

within all the selected fields, the 'Z' pattern sampling method previously used by many others (e.g. Gul *et al.*, 2013) was used. In square shaped crops fields W or U shaped sampling patterns are usually used and in rectangular field Z or zig-zag pattern of sampling is commonly more reliable and efficient. In this method we can sample more number of plants easily and efficiently (Zehnder, 2010). The field sites selected in Peshawar division (Regi, Warmarh and Pul Dehri Mehra), Malakand division (Miandam valley, Kalam and Malamjaba) and Hazara (Kaghan, Bagnotar and Kalandarabad). The potato cultivars in selected and sampled farmer's fields in Malakand division were Cardinal, Kurada and Sahiwal Red, in Peshawar division Desiree, Dura and Sarpo Mira and in Hazara division Rocco, Diamond and Desiree. One sample size consisted of three leaves per plant (manually cut from the top, middle and bottom) of potato plants. All the cut samples were separately placed in polythene zip-lock bags printed with regions, field sites, sample number and samples collection dates. The results of ELISA is more reliable and exhibited higher infection compared to visual inspection of potato crop. The results showed from the current experiment that potato virus Y and potato leafroll virus can affect the quality and production of potato in Khyber Pakhtunkhwa Pakistan. All collected samples were taken to the Virology Laboratory, Department of Plant Pathology, The University of Agriculture, Peshawar, Pakistan. As it was difficult to complete the serological test for all the samples immediately in a single day, samples were preserved in a refrigerator ( $4 \pm 1^\circ\text{C}$ ) to avoid any possible viral infection loss for further testing.

#### Serological detection test

All potato samples collected from the three different areas (Peshawar, Malakand and Hazara divisions) of Khyber Pakhtunkhwa province were tested for detection of PVY and PLRV infection by the DAS-ELISA methodology using antibodies (Bioreba Kit). The ELISA is one of the most reliable and a highly sensitive detection method of a number of morphologically different viruses in purified preparations and in unclarified infected crop plants. This method is capable to detect and quantify both the target antigen and molecules in study samples using a specific using an enzyme (peroxidase) reaction with its substrate. Monoclonal antibodies (IgG) were diluted in coating buffer (at  $1\mu\text{L}:500\mu\text{L}$ ) and 10 plates

of ELISA (each plate having 96 wells) were coated with diluted IgG ( $100\mu\text{L}/\text{well}$ ) separately for each of the two viruses PVY and PLRV. The coated plates were air tight by aluminium foil and incubated for 3 hours at room temperature. Potato leaves were ground using pestle and mortar in extraction buffer (Polyvinylpyrrolidone (PVP), albumin and Tween-20) to create sap, filtered through muslin cloth, sap was filled in well (each well being filled with  $100\mu\text{L}$  antigen sap) and a control (containing healthy control). The antigen coated plates were incubated over night c. 14 hours at  $4^\circ\text{C}$  while enzyme alkaline phosphatase (ALKP) conjugated IgG was diluted (1:500) in conjugate buffer and was added in each well. Each step of the test was completed at 3 minutes interval and buffer and plates were dried by using paper towels. Substrate buffer ( $150\mu\text{L}$ ) containing p-nitro phenyl phosphate ( $1\text{mgmL}^{-1}$ ) was added to each well and all plates were incubated in dark for 1 hour and the color reaction was observed visually. A sample was considered and noted to be virus infected when the absorbance of 405 nm was at least three times greater than the healthy control. The incidence of potato viruses (PVY and PLRV) was calculated by the formula:

$$\text{Virus incidence (\%)} = \frac{\text{Number of infected samples}}{\text{Total samples}} \times 100$$

#### Statistical analysis

The recorded data, in all the three regions and within each of the selected potato grown field for survey on PVY and PLRV were tested for proportion difference by using Z-test. Before applying the Z-test, the proportion of positives and negatives for PVY and PLRV were computed, and it was considered that there is no significance difference between the proportion of positives of PVY and PLRV, in each of the selected surveyed region. All the results were described at 5% level of probability.

## Results and Discussion

The results in Table 1 show the region-wise relative incidence and distribution of *M. persicae* borne viruses PVY and PLRV. The proportional difference between the PVY and PLRV in Malakand region was recorded to be 0.180, in Peshawar region 0.147 and in Hazara region 0.133 (Table 1). The Z-test values recorded for PVY and PLRV in the three selected regions were



**Table 1:** The proportional incidence and distribution of *M. persicae* borne viruses PVY and PLRV in three selected regions in Khyber Pakhtunkhwa, Pakistan.

| Regions  | PVY                  |                  | PLRV                 |                  | Proportion difference | S.E   | Z-test values | P value |
|----------|----------------------|------------------|----------------------|------------------|-----------------------|-------|---------------|---------|
|          | Total samples tested | Positive samples | Total samples tested | Positive samples |                       |       |               |         |
| Malakand | 150                  | 94               | 150                  | 67               | 0.180                 | 0.058 | 3.01          | 0.0026  |
| Peshawar | 150                  | 83               | 150                  | 61               | 0.147                 | 0.058 | 2.43          | 0.0152  |
| Hazara   | 150                  | 76               | 150                  | 56               | 0.133                 | 0.057 | 2.21          | 0.0271  |

3.01 2.43 and 2.21 for Malakan, Peshawar and Hazara, respectively. The *P*-value of proportional differences between PVY and PLRV in the three selected regions were Malakand - 0.0026, Peshawar-0.0152 and Hazara-0.0271 (Table 1). This experiment was undertaken with main focus on detecting incidence of potato viruses including PVY and PLRV in potato in three major growing areas in Malakand, Peshawar and Hazara, Khyber Pakhtunkhwa, Pakistan. The presence of the two major viruses PVY and PLRV in potato plants in Khyber Pakhtunkhwa, Pakistan can be found and as has been reported in many earlier studies (e.g. Abbas *et al.*, 2016; Abbas and Amrao, 2017). In above Chapters (3, 4 and 5) a number of potato cultivars (e.g. Sahiwal Red, Sarpo Mira and Rocco) shown resistance against *M. persicae* under glasshouse and field conditions. *Myzus persicae* being an important vector of viruses, the resistant potato cultivars may have an impact on the transmission of viruses in potato crop in field conditions.

**Table 2:** The region-wise incidence and distribution of *M. persicae* transmitted Viruses PVY and PLRV in potato crop in Peshawar, Malakand and Hazara divisions of Khyber Pakhtunkhwa, Pakistan.

| Region-wise PVY  | Total samples tested | Total samples (+ve) | Incidence (%) |
|------------------|----------------------|---------------------|---------------|
| Malakand         | 150                  | 94                  | 62            |
| Peshawar         | 150                  | 83                  | 55            |
| Hazara           | 150                  | 76                  | 50            |
| Region-wise PLRV |                      |                     |               |
| Malakand         | 150                  | 67                  | 44            |
| Peshawar         | 150                  | 61                  | 40            |
| Hazara           | 150                  | 56                  | 37            |

The Table 2 shows the incidence of PVY and PLRV in potato crop in survey fields in Malakand, Peshawar and Hazara regions. The results showed the incidence of PVY to be the highest in Malakand division (62%), followed by the Peshawar region (55%) and the lowest for Hazara region (50%). The incidence of PLRV

ranged from 37 – 44 % among the three regions. The maximum incidence were recorded in Malakand region (44%), followed by Peshawar region (40%) whereas, the minimum incidence was recorded in Hazara region (37%) Table 2. The ELISA based results showed the incidence of both PVY and PLRV in potato crop in all the three surveyed regions, however, the incidence of PVY was widely spread followed by the relatively scattered incidence of PLRV in potato grown fields in all the three regions (Tables 2 and 3). Interestingly the results showed low incidence of PVY and PLRV in fields cultivated with Sahiwal red and Rocco in sampled fields in all the three surveyed divisions while high incidence of the two viruses in fields cultivated with potato cultivars such as Desiree and cardinal (Tables 3 and 4). The diversified levels of incidence of PVY and PLRV detected in potato cultivated fields in the three regions may be due to the diverse resistance capabilities of the potato cultivars against the viruses under field conditions. A number of potato cultivars tested both under glasshouse and field conditions have shown resistance to *M. persicae* (Tables 1, 2 and 1-4 in Chapter 3 and 5). The lower incidence of PVY and PLRV detected in some of the potato cultivars e.g. Sarpo Mira and Rocco (48 and 44% and 42 and 22%, respectively) may have been due to the no-to-limited attack of *M. persicae* on plants of such potato cultivars and may have had restricted the transmission of the viruses in potato plants. In earlier studies (e.g. Nikan and Barker, 2012; Hameed *et al.*, 2014) varied incidence of potato viruses was detected under field conditions due to either resistance of the potato cultivars to the viruses or due to the resistance of the potato cultivars to the *M. persicae*. The difference in the incidence of PVY and PLRV in potato cultivars could also be due to the agro-climatic conditions prevailing in the three surveyed regions when other abiotic and biotic aspects come into play. Agro-environmental conditions of an area have been reported to play important role in viruses incidence in potato (Roossinck, 2015; Bertschinger *et al.*, 2017; Makarova *et al.*, 2018).

**Table 3:** The field-wise incidence and distribution of *M. persicae* borne viruses PVY and PLRV in Malakand, Peshawar and Hazara regions of Khyber Pakhtunkhwa, Pakistan in 2016.

| Fields selected        | Potato cultivar | Samples tested | PVY | Incidence (%) | PLRV | Incidence (%) |
|------------------------|-----------------|----------------|-----|---------------|------|---------------|
| <b>Malakand region</b> |                 |                |     |               |      |               |
| Miandam valley         | Cardinal        | 50             | 31  | 62            | 23   | 46            |
| Kalam                  | Kurada          | 50             | 35  | 70            | 25   | 50            |
| Malamjaba              | Sahiwal Red     | 50             | 28  | 56            | 19   | 38            |
| <b>Peshawar region</b> |                 |                |     |               |      |               |
| Regi                   | Dura            | 50             | 26  | 52            | 16   | 32            |
| Warmarh                | Desiree         | 50             | 33  | 66            | 23   | 46            |
| Pul dehri Mehra        | Sarpo Mira      | 50             | 24  | 48            | 22   | 44            |
| <b>Hazara region</b>   |                 |                |     |               |      |               |
| Kaghan                 | Diamond         | 50             | 31  | 62            | 27   | 54            |
| Kalanderabad           | Rocco           | 50             | 21  | 42            | 11   | 22            |
| Bagnetar               | Desiree         | 50             | 24  | 48            | 18   | 36            |

The results showed that the incidence and distribution of PVY and PLRV in Malakand region to be ranged from 56-70 and 38-50%, respectively (Table 3). The highest incidence of PVY was noted in potato crop in Kalam (70 %) followed by the potato crop in Miandam (62%) and the lowest incidence of PVY in Malamjaba (56%) of Malakand region. Similarly, the incidence of PLRV was highest in Kalam (50%) followed by Miandam (46%) and lowest incidence was recorded in Malamjaba (38%; Table 3). The Peshawar region had the maximum PVY incidence in potato crop in Warmarh (66%) followed by the potato crop in Regi (52%) and the lowest incidence were found in Pul dehri Mehra (48%). Similarly, the incidence of PLRV were recorded maximum in Warmarh (46%) followed by Pul dehri Mehra (44%) and the minimum incidence of PLRV were recorded in Regi (32%) Table 3. The Hazara region had the highest PVY incidence in potato crop in Kaghan (62%) followed by the potato crop of Bagnetar (48 %) and the minimum incidence were noted in Kalanderabad (42%). Similarly, the incidence of PLRV were recorded maximum in Kaghan (54 %) followed by Bagnetar (36 %) and the minimum incidence of PLRV were recorded in potato crop of Kalanderabad (22%) Table 3. The serological test detected the presence of PVY and PLRV in different surveyed regions of Khyber Pakhtunkhwa, Pakistan. Among the two viruses, PVY showed highest incidence in all the three regions as compared to the PLRV (Tables 2 and 3). The greater incidence of PVY in surveyed regions could be due to its more successful transmission by different vectors and or by different mechanical means. Earlier studies revealed that PVY had more infestations compared to

other viruses such as PVS, PLRV and PVM (Gul et al., 2013). Results showed that Kalam valley in Malakand region, Kaghan valley in Hazara region and Warmarh in Peshawar region to show greater incidence of PVY while Malamjaba region in Malakand, Kalanderabad region in Hazara and Pul dheri Mehra region in Peshawar had the least incidence of the virus (Table 3). The diverse incidence of viruses in sampled locations within the three regions may be due to the different agro-climatic conditions as discussed in above lines.

The result shows the field wise proportional distribution of *M. persicae* borne viruses PLRV and PVY. The proportional difference between the two viruses (PLRV and PVY) in Malakand region were recorded in the three different fields (0.160) in Kalam, (0.200) in Miandam valley and in Malamjaba (0.180) Table 4. In Peshawar region (0.200) in Regi and Warmarh, while the (0.040) were recorded in Pul dehri Mehra, Table 4. The proportional difference between PVY and PLRV among three different fields of Hazara were recorded as (0.080) in Kaghan, (0.200) in Kalanderabad and (0.120) in Bagnetar, Table 4. The z test and p values were recorded for the two viruses and three different regions and different fields are (1.40, 0.1602) Kalam, (1.60, 0.0662) Miandam valley and (1.60, 0.1090) for Malamjaba were recorded for Malakand region, respectively. The z test and p values for Peshawar region were recorded for the two viruses and three different fields are (1.82, 0.0682) Regi, (1.81, 0.0698) Warmarh and (0.20, 0.8410) for Pul dehri Mehra were recorded, respectively Table 4. The z test and p values for Hazara region were recorded for the two viruses and three different fields are

**Table 4:** The proportional incidence and distribution of *M. persicae* borne viruses PVY and PLRV in three selected regions selected fields in Khyber Pakhtunkhwa, Pakistan.

| Fields             | Potato cultivar | Samples tested | PVY | PLRV | Proportion difference | S.E   | Z-test values | P value |
|--------------------|-----------------|----------------|-----|------|-----------------------|-------|---------------|---------|
| Miandam valley     | Cardinal        | 50             | 31  | 23   | 0.160                 | 0.099 | 1.40          | 0.1602  |
| Kalam              | Kurada          | 50             | 35  | 25   | 0.200                 | 0.098 | 1.84          | 0.0662  |
| Malamjaba          | Sahiwal red     | 50             | 28  | 19   | 0.180                 | 0.099 | 1.60          | 0.1090  |
| Regi               | Dura            | 50             | 26  | 16   | 0.200                 | 0.098 | 1.82          | 0.0682  |
| Warmarh            | Desiree         | 50             | 33  | 23   | 0.200                 | 0.099 | 1.81          | 0.0698  |
| Bridge dehri Mehra | Sarpo Mira      | 50             | 24  | 22   | 0.040                 | 0.099 | 0.20          | 0.8410  |
| Kaghan             | Diamond         | 50             | 31  | 27   | 0.080                 | 0.099 | 0.61          | 0.5433  |
| Kalanderabad       | Rocco           | 50             | 21  | 11   | 0.200                 | 0.093 | 1.93          | 0.0537  |
| Bagnetar           | Desiree         | 50             | 24  | 18   | 0.120                 | 0.099 | 1.01          | 0.3110  |

(0.61, 0.5433) Kaghan, (1.93, 0.0537) Kalanderabad and (1.01, 0.3110) for Bagnetar were recorded, respectively Table 4. In previous studies (Gul *et al.*, 2013; Attaullah and Arif, 2017) different extent of PVY and PLRV incidences were reported in major potato growing areas in Khyber Pakhtunkhwa province of Pakistan. Potato virus Y and Potato leafroll virus reduce yields of the crop, in the case when the disease is caused through tuber seeds or transmitted via vector insects (Rykbost *et al.*, 1999; Nolte *et al.*, 2004). Taken as a whole the incidence of PVY was found to be higher than that of the PLRV in the potato in the all the three surveyed regions (Tables 2 and 3). The differential extent of PVY and PLRV may have been linked to a number of factors of which one may be the adaptability of the viruses to the agro-climatic conditions as discussed above and the second most important factor could be the degree of difference in resistance capability of the potato cultivars sown in the surveyed regions. Both these factors have been largely reported to play role in restricting incidence of aphids and diseases (Batool *et al.*, 2011; Valkonen, 2015).

## Conclusions and Recommendations

The results obtained revealed the incidence of both PVY and PLRV but with different degree of their incidence in Malakand, Peshawar and Hazara divisions where PVY being the dominant virus in most of the surveyed fields of potato. The difference in the incidence of the PVY and PLRV could be due to a number of factors as has been discussed above. Nevertheless incidence of these viruses of potato has been reported to hold an important position in reducing the potato yield in Khyber Pakhtunkhwa

(Malko *et al.*, 2019).

This present study did not measure the yield losses by the PVY and PLRV to the potato crop in the surveyed areas, future research could be useful to measure the exact yield losses as well as the tuber quality deterioration by the viruses.

## Novelty Statement

Potatoes crop is very important crop worldwide. White aphid is serious pest to this crop. This study was conduct to screen out selected to screen out potatoes genotypes for their control of virus dictation through ELISA against potato aphid infestation under laboratory condition.

## Author' Contribution

Noor Muhammad: conception and design.

Shah Alam Khan and Naeem khan: drafting the article.

Iqbal Munir: analysis and interpretation of the data; Tamana Bakht: revising it critically for important intellectual content and

Luqman and Zafrullah Khan: helping in the correction of whole text

Ruqayya Qazi and Shazia Khushdil: helped in data collection.

The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript

## Conflict of interest

The authors have declared no conflict of interest.

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