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Int. J. Phytopathol. 10 (02) 2021. 141- 154 DOI: 10.33687/phytopath.010.02.3781

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Available Online at EScience Press
International Journal of Phytopathology
ISSN: 2312-9344 (Online), 2313-1241 (Print)
https://esciencepress.net/journals/phytopath

DETECTION OF MAJOR SOIL-BORNE VIRUSES AND ASSESSMENT OF VIRUS-VECTOR ASSOCIATION IN POTATO GROWING AREAS OF NORTH-WESTERN PAKISTAN (KHYBER PAKHTUNKHWA) AND AZAD JAMMU AND KASHMIR

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ARTICLE INFO

Article history
Received: June 11, 2021
Revised: August 19, 2021
Accepted: August 28, 2021

Keywords
Potato
Incidence
PMTV
TRV
Spongospora subterranean
Trichodorus
Paratrichodorus
Association

ABSTRACT

Soil-borne potato viruses are an increasing economic threat to crop yield in the future. *Potato mop-top virus* (PMTV) and *Tobacco rattle virus* (TRV) and their vector association in field infection in North-western region of Pakistan and Azad Jammu and Kashmir were surveyed in this study. Incidence and distribution of these soil-borne viruses were estimated by field sample collection from Malakand and Hazara divisions of KPK and selected areas of Azad Kashmir. PMTV incidence was 22% in Malakand (highest of 40% in Swat II and Swat V), 24% in Hazara (highest 50% in Galliyat), and 23% from selected areas of Azad Jammu and Kashmir (AJK) (Highest with 40% in Rawalakot and Trarkhal). TRV incidence was 49% in AJK (100% highest field incidence in Khaigala Rawalakot), 53% in Malakand division (80% highest field incidence in Swat II), and 24% in Hazara division (70% highest field incidence in Abbottabad and Mansehra districts). PMTV, TRV vectors are *Spongospora subterranea* and *Trichodorus*, *Paratrichodorus* spp. were found distributed in all surveyed areas. Eighty percent (80%) fields were found infested with *S. subterranean, while Trichodorus and Paratrichodorus were found in 64%* in Malakand. In Hazara, 84% of surveyed fields were found infested with S. subterranea while 65% were found to be infested with *Trichodorus, Paratrichodorus*. In AJK *S. subterranea* was found distributed in 79% of fields. A significant relationship (P value=0.000) between viruses (PMTV and TRV) and their respective vectors were detected in proportionate. The corresponding R^2 (0.70 and 0.82) indicates positive relation between viruses and their vectors. Positive Pearson correlation was found among incidence and severity of virus infection (PMTV and TRV) and vectors (*S. subterranea*; *Trichodorus minor*, *Paratrichodorus*), indicating increased disease severity with vector presence and activity. The study will be a tool in vector virus management to economical potato harvest.

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INTRODUCTION

Potato (*Solanum tuberosum*) is an important crop that belongs to the family Solanaceae, genus *Solanum*. It is cultivated in temperate, tropical, and subtropical regions (Hawkes, 1990; Stevenson *et al.*, 2001).

Potential biotic threats include fungi, nematodes, bacteria, viruses, viroids, phytoplasmas, and insect pests to potato crop. Viruses are among important pathogens that are transferred via diverse vectors (Jeffries *et al.*, 2005; Valkonen, 2007). Plant-parasitic nematodes are found infesting vegetable crops in Azad Jammu and Kashmir (Tariq-Khan *et al.*, 2017; Tariq-Khan *et al.*, 2020c; Tariq-Khan *et al.*, 2020a; Tariq-Khan *et al.*, 2020b) including endo-parasitic and ecto-parasitic root-knot nematodes. *Potato mop-top virus* (PMTV) and *Tobacco rattle virus* (TRV) are transmitted by *Spongospora subterranea* and various species of soil-borne plant pathogenic nematode groups *Trichodorus* and *Paratrichodorus.* PMTV is type species of genus *Pomovirus*; family *Virgaviridae* (King *et al.*, 2011) and is responsible for spreading disease in potato tubers (Harrison and Reavy, 2002). *Spongospora subterranea* incites powdery scab disease of potato, has been reported as PMTV vector (Jones and Harrison, 1969; Hims, 1975; Arif *et al.*, 1995). It acts not only as an obligate parasite but vector of PMTV in potato crop. Resting fungal spores can survive for many years in the soil without a suitable plant host (Jones and Harrison, 1969; Harrison and Jones, 1970; Arif *et al.*, 1995; Merz, 1995; Campbell, 1996). Major symptoms are brown circles on tuber surface and arcs in flesh of susceptible potato cultivars with reduced aesthetic value (Kurppa, 1989; Sandgren, 1995; Sandgren *et al.*, 2002). The temperature in the range of 12-20˚C is conducive to infect potato tubers and provides the base for host selection (Carnegie *et al.*, 2010).

TRV is a *Tobravirus* and is considered important soil-borne potato virus (Robinson and Harrison, 1989), resulting in corky ring-spot in potato. TRV has rigid rod-shaped particles with two types; M type, and NM type, based on their genomic makeup (Nicolaisen *et al.*, 1999). TRV causes corky ring-spot in potato. The primary symptoms of this disease are internal discoloration which sometimes leads to arcs or circles in potato tuber flesh. The infested tissues have corky texture and symptoms visible on tuber surface and ringspot inside tuber flesh (Harrison, 1970) reducing aesthetic value. Stubby-root nematodes are soil-borne vector of TRV are polyphagous, and feed on from the roots of plants as migratory ecto-parasites. Initially, root becomes stunted then growth of roots is reduced due to direct damage by trichodorid nematodes (Harrison and Robinson, 1986).

Soil-borne viruses are important for seed and storage potato production in Pakistan. Little work has been done to detect and identify soil-borne potato viruses (Rehman, 2013; Arif *et al.*, 2014). Major soil-borne viruses and their association with vectors are still missing to be explained for potato crop. This study explains the incidence, distribution, and virus vector association of soil-borne viruses (PMTV and TRV) from potato crop cultivated from various locations of the North-western region of Pakistan and Azad Jammu and Kashmir.

MATERIAL AND METHODS

Collection of potato tubers and soil samples for detection of Pathogens and their vectors

Potato tubers and soil samples were collected from 24 locations of Malakand and Hazara divisions, Northwestern Khyber Pakistan, and from selected potato growing areas of Azad Jammu and Kashmir during March 2015. The fields were sampled in zigzag pattern, and soil samples were taken from 20-30 cm (Whitehead and Hooper, 1970). One kg composite soil sample from one location was collected, packed in polyethylene bags with some addition of freshwater for nematodes survival, and taken to the Nematology lab at Department of Plant Pathology, The University of Agriculture, Peshawar, and stored at 4 ℃. The sampled potato tubers from 24 locations were kept at room temperature.

Assessment of PMTV and TRV infection in potato tubers based on symptoms

Potato tubers were assessed for the external symptoms of PMTV and TRV and cut into two halves, and data of internal symptoms were recorded by comparing scale 0-4 (Table 1) (Robinson and Harrison, 1989). The percent incidence of PMTV and TRV and their vectors (*S. subterranea* and *Trichodorus, Paratrichodorus*) was determined by using formula given below (Allen, 1983).

$$Incidence = \frac{Infested\ Tubers}{Total\ Number\ of\ tested\ Tubers} \times 100$$

Detection and Assessment of PMTV and TRV infection in Potato tubers

Potato mop-top virus has been detected and identified based of external and internal symptoms on tubers and by DAS-ELISA. DAS–ELISA was performed by coating

with conjugate antibodies (Adgen Phyto-diagnostics, UK). The tests were performed in polystyrene micro-plates (NUNC, Immunoplate II, Thermal scientific, MA, USA) (Clark and Adams, 1977; Arif *et al.*, 2014).

Table 1: Scale designed, modified, and used for the assessment of severity of *Potato mop-top virus* and *Tobacco rattle virus* in potato tubers.

Scale	Description	Mean A_{405nm} value (PMTV)	Mean A_{405nm} value (TRV)
0	No reaction apparently healthy	0.140	0.140
1	Positive (hardly visible reaction)	0.350	0.389
2	Positive (quite visible reaction)	0.745	0.750
3	Positive (strong reaction)	1.245	1.250
4	Positive (very strong intense reaction)	1.750	1.750

Assessment of powdery scab infection in potato tubers

Tuber samples were visually examined for the powdery scab infection using an assessment key (Iftikhar *et al.*, 2007; Nakayama *et al.*, 2007).

Extraction and identification of nematode vector from soil

The nematodes were extracted using the Modified Baermann technique (Whitehead and Hemming, 1965) from 100 g fine soil by placing it on tissue paper on coarse sieve in a tray filled with fresh water. Tray will be kept at room temperature for maximum downward movement of nematode fauna for 24-36 h for maximum nematode harvest. The soil in sieves was removed and discarded. Water suspension containing nematodes was transferred to sterilized glass beakers and allowed to settle for 3-4 hours. Extra water was removed by siphoning, and collected settled nematodes were put into counting dish for population estimation. Nematode suspensions were passed through 32 µm, and 25 µm mesh size sieves for maximum number of nematodes (*Trichodorus* and *Paratrichodorus*). Nematodes extracted were counted under stereoscopic microscope (6X magnification), and their population density was determined using method (Whitehead and Hooper, 1970).

Detection of TRV in nematode vector

To confirm viruliferous nematode, ten nematodes per sample were picked using dropper and put into an eppendorf tubes with 2 ml virus-specific extraction buffer. The nematodes were centrifuged at 8,000–10,000 rpm for 5-10 min, then re-suspended the pellet into 0.5 ml of extraction buffer followed by frozen at -80 ℃ for 30 min, then thawed on ice and used for ELISA detection (Arif *et al.*, 2014).

Assessment of the association between soil-borne viruses and their vectors

Association of soil-borne viruses (PMTV and TRV) and their vectors *S. subterranean*; *Trichodorus*; *Paratrichodorus was* assessed with the Jaccard Similarity Index (J) as described by Montero-Astúa *et al.* (2008). Linear regression was performed to test the significance of association of the virus (PMTV and TRV) with *S. subterranean, Trichodorus* and *Paratrichodorus*) respectively. The relationship between incidence and severity was computed by Pearson correlations using Statistics 8.1 package (Öfversten, 1995).

RESULTS

Symptom based identification and DAS-ELISA confirmation of PMTV (*Potato Mop Top Virus*)

PMTV exhibited necrotic rings on tuber surface and necrotic lesions as brown arc in tuber flesh. PMTV infected potato tubers of susceptible cultivars planted in the field were found with cracks and distortion. The average incidence of PMTV in Malakand based on external and internal symptoms on tuber was 24% and 20%, respectively, while in Hazara, it was estimated as 23% and 24.4%, respectively (Table 2) and average estimated incidence in Azad Jammu and Kashmir was 20% in external and 26%. The results were further confirmed by the detection of PMTV in potato tubers using DAS-ELISA. The A_{405} nm value of positive samples was relatively lighter than the three times mean values of healthy control. PMTV was detected in 12 out of 50 tuber samples tested from

Malakand division, while 11 out of 45 were from Hazara division. PMTV was also detected in 5 out of 25 tubers from Azad Kashmir (Table 3).

Symptom-based identification and DAS-ELISA confirmation of TRV

Potato tuber exhibited symptoms like raised necrotic rings, corky ring spots on tuber surface, and multiple necrotic flecks/lines in tuber flesh as internal symptoms with 53 and 54% tubers, respectively. In Hazara division the average incidence of TRV was 52% and 46%, while highest (70%) and lowest (30%) incidence was observed in Abbottabad and Mansehera districts. In Azad Kashmir, 20% incidence was observed externally on tubers and 78% internally (Table 2). TRV confirmed with DAS-ELISA in potato tubers of almost all surveyed areas, the incidence was highest 44% in Malakand division, followed by 42% to Hazara division and 36% from Azad Jammu and Kashmir (Table 4).

Table 2: Detection of major soil-borne viruses of potato in Northwest of Pakistan on the basis of external and internal symptoms.

Division/Region	District	Location	Detection of soil-borne viruses			
			PMTV (%)		TRV (%)	
			External sym on tuber[1]	Internal sym on tuber[2]	External sym on tuber[3]	Internal sym on tuber[4]
Malakand	Shangla	Shangla I	10	10	40	60
		Shangla II	10	10	50	60
		Shangla III	30	20	60	60
		Shangla IV	30	20	60	60
	Swat	Swat I	10	10	50	50
		Swat II	40	40	80	60
		Swat III	30	20	60	60
		Swat IV	20	10	30	50
		Swat V	40	40	50	40
		Swat VI	20	20	50	40
Average incidence			24	20	53	54
Hazara	Abbottabad	Galliyat	50	40	60	60
		Namlimaira	20	10	60	60
		Sajikot	40	40	60	60
	Manshera	Mangal	10	10	70	70
		Shinkiari	20	30	30	40
		Parhana	10	20	40	30
		Khaki	40	40	70	60
		Baffa	10	20	30	30
		Bajna	10	10	50	40
Average incidence			23	24.4	52	46
Azad Kashmir	Poonch	Rawalakot	40	40	30	60
		Khaigala	0	10	20	100
		Chottagala	10	20	10	70
		Banjonsa	10	20	30	80
	Sudhnoti	Trarkhal	40	40	10	80
Average incidence			20	26	20	78

Disease rating scale is given in Table 1; sym: Symptoms

Assessment of association of PMTV with *S. subterranea* using Jaccard Similarity Index (JSI)

Significant association of PMTV and *S. subterranea* was computed in field conditions (Table 5). PMTV and *S. subterranean* computed association 12.2% found in Malakand division from 120 samples, highest 20% in district Swat and lowest 8.3% in district Shangla. In Hazara division, overall computed association was 4.6% from 285 samples, highest 9.1% in Khaki, and no association was found in Parhana, district Manshera. In Azad Jammu and Kashmir JSI was 5.3% from 117 samples, highest 22.2% in Trarkhal, and no association was detected in Banjonsa and Khaigala (Table 5).

Table 3: Incidence and severity of *Potato mop-top virus* and *S. subterranea* in selected areas of Northwest of Pakistan.

Division/Region	District	Location	Incidence[1]		Severity[2]	
			PMTV (%)	*S. subterranea* (%)	PMTV	*S. subterranean*
Malakand	Shangla	Shangla I	20	71	3	2
		Shangla II	20	65	2	2
		Shangla III	20	80	2	2
		Shangla IV	20	82	3	2
	Swat	Swat I	20	77	2	2.5
		Swat II	40	92	2	2
		Swat III	20	87	3	1.5
		Swat IV	20	71	2	2.5
		Swat V	40	100	2	3.5
		Swat VI	20	80	2	2.5
Average incidence			24	80		
Hazara	Abbottabad	Galliyat	40	100	2	3
		Namlimaira	20	77	3	2.5
		Sajikot	40	96	2.5	3
	Manshera	Mangal	20	80	2	4
		Shinkiari	20	80	2	3.5
		Parhana	0	71	0	2.5
		Khaki	40	100	2.5	3.5
		Baffa	20	77	3	4
		Bajna	20	72	3	3
Average incidence			24.4	84		
Azad Kashmir	Poonch	Rawalakot	40	100	1.5	3.5
		Khaigala	0	50	0	3
		Chottagala	20	66	3	3
		Banjonsa	0	78	0	3.5
	Sudhnoti	Trarkhal	40	100	1.5	3
Average incidence			20	79		

Disease Severity Scales:

0= 0.140 (No reaction apparently healthy)
1= 0.350(Positive hardly visible reaction)
2=0.745 (Positive quite visible reaction)
3= 1.245 (Positive strong reaction)
4= 1.750 (Positive very strong intense reaction)

1. *Potato mop top virus* (PMTV)
2. S. *subterranea* 0 = No infection; 1= Less than 1%; 2= 1-10%; 3= 11-20%; 4=21-50%; and 5= 51% and more area infested respectively

Table 4: Incidence and severity of *Tobacco rattle virus* and vector nematode in selected areas of Northwest of Pakistan.

Division/Region	District	Location	Incidence[1]		Severity[2]	
			TRV (%)	Nematode vector (%)	TRV	Nematode Vector
Malakand	Shangla	Shangla I	40	60	3	2
		Shangla II	40	65	3	2
		Shangla III	60	70	2	-
		Shangla IV	60	70	2	2
	Swat	Swat I	40	62	2	-[3]
		Swat II	60	75	1	2
		Swat III	40	64	3	-
		Swat IV	20	50	2	-
		Swat V	40	68	3	3
		Swat VI	40	60	2	-
Average incidence			44	64		
Hazara	Abbottabad	Galliyat	60	72	1	-
		Namlimaira	40	67	2	3
		Sajikot	40	64	2	2
	Manshera	Mangal	60	78	3	-
		Shinkiari	20	55	2	3
		Parhana	40	58	2	-
		Khaki	60	70	2	2
		Baffa	20	54	3	3
		Bajna	40	65	2	2
Average incidence			42	65		
Azad Kashmir	Poonch	Rawalakot	40		2	*[4]
		Khaigala	40		3	*
		Chottagala	40		3	*
		Banjonsa	60		1	*
	Sudhnoti	Trarkhal	0		0	*
Average incidence			36			

Severity Scale of TRV and vector nematode is
0= 0.140 (No reaction apparently healthy);
1= 0.389 (Positive hardly visible reaction);
2= 0.750(Positive quite visible reaction);
3= 1.250 (Positive strong reaction);
4= 1.750 (Positive very strong intense reaction).
Locations where vector severity were not identified * Locations where vector severity were not tested

Assessment of association of TRV and *Trichodorus, Paratrichodorus spp.* using JSI

Positive interaction between TRV and its nematode vector was found (Table 6), with highest association 30% each in two districts of Malakand and 33.3% in Namlimaira (Abbottabad) in Hazara. In comparison, the lowest association (9.1%) was observed in Swat IV Malakand, 16.6% in Parhana and Baffa Hazara. The JSI value for 100 samples from Malakand was 21.8% and 90 samples from Hazara were 22.6% (Table 6).

Table 5: Association of PMTV and *S. subterranea* using Jaccard Similarity Index.

Division	District	Location	No. of samples	PMTV/S. subterranea[2]			Jaccard Index
				a +/+	b+/-	c -/+	
Malakand (KPK)	Shangla	Shangla I	14	1	1	9	9.1
		Shangla II	14	1	0	8	11.1
		Shangla III	15	1	0	11	8.3
		Shangla IV	11	1	0	8	11.1
	Swat	Swat I	13	1	0	9	10.0
		Swat II	12	2	1	9	16.6
		Swat III	8	1	0	6	14.3
		Swat IV	14	1	0	9	10.0
		Swat V	9	2	1	7	20.0
		Swat VI	10	1	1	7	11.1
Total			120	12	4	83	12.2
Hazara (KPK)	Abbottabad	Galliyat	30	2	1	28	6.5
		Namlimaira	31	1	1	23	4.0
		Sajikot	28	2	1	25	7.2
	Manshera	Mangal	25	1	0	19	5.0
		Shinkiari	56	1	0	44	2.2
		Parhana	28	0	0	20	0.0
		Khaki	21	2	1	19	9.1
		Baffa	30	1	0	22	4.4
		Bajna	36	1	0	25	3.8
Total			285	11	4	225	4.6
Azad Jammu and Kashmir (AJK)	Poonch	Rawalakot	40	2	1	38	4.8
		Khaigala	20	0	0	10	0.0
		Chottagala	35	1	0	22	4.4
		Banjonsa	14	0	0	11	0.0
	Sudhnoti	Trarkhal	8	2	1	6	22.2
Total			117	5	2	87	5.3

[1] Data of virus incidence was based on ELISA detection.

[2] Values showing positive (+) and negative (-) test results for *Potato mop top virus*

$$\text{Jaccard Index } (S.\,subterranea) = \frac{a}{(a+b+c)} \times 100$$

Where a = the number of samples where both the virus and vector present. b = the number of samples where the virus present but the vector was absent. c = the number of samples where the vector present but the virus was absent.

Assessment of association between PMTV and *S. subterranea* using regression analysis

The association between PMTV incidence and *S. subterranea* incidence has been determined, which is highly significant (P-value= 0.000) with 0.83 co-efficient of determination 0.83. It is determined that 1% increase in incidence of *S. subterranea* in field increase PMTV incidence 0.97% (Figure 1). The relationship between PMTV and *S. subterranea* incidence on internal symptoms in potato tubers revealed positive relationship with 0.73 R^2 (Figure 2) value. Further, when the incidence of vector under field conditions increases by 1%, the incidence of PMTV increases by 0.79%. The regression analysis for the vector and PMTV based on ELISA (Figure 3) shows R^2 value as 0.70. The relationship between incidence of PMTV and vector were found to be statistically significant at (P-value= 0.000). Under field conditions, when the population of *S. subterranea* increases by 1% it increases 0.80% PMTV incidence.

Table 6: Association of TRV and nematode vector using Jaccard Similarity Index.

Division/ Region	District	Location	No. of samples	TRV/ Nematode vector[2]			Jaccard Index
				a +/+	b +/-	c -/+	
Malakand	Shangla	Shangla I	10	2	0	6	25.0
		Shangla II	10	2	0	6	25.0
		Shangla III	10	3	1	6	30.0
		Shangla IV	10	3	1	6	30.0
	Swat	Swat I	10	2	1	10	15.4
		Swat II	10	3	1	6	30.0
		Swat III	10	2	0	10	16.6
		Swat IV	10	1	0	10	9.1
		Swat V	10	2	1	4	28.6
		Swat VI	10	2	0	10	16.6
Total			100	22	5	74	21.8
Hazara	Abbottabad	Galliyat	10	3	1	10	21.4
		Namlimaira	10	2	0	4	33.3
		Sajikot	10	2	0	6	25.0
		Mangal	10	3	1	10	21.4
		Shinkiari	10	1	0	4	20.0
		Parhana	10	2	0	10	16.6
	Manshera	Khaki	10	3	1	6	30.0
		Baffa	10	1	1	4	16.6
		Bajna	10	2	1	6	22.2
Total			90	19	5	60	22.6

[1] Data of virus incidence was based on ELISA detection.

[2] Values showing positive (+) and negative (-) test results for *Potato mop top virus*

$$\text{Jaccard Index } (S.\,subterranea) = \frac{a}{(a+b+c)} \times 100$$

Where;

a = the number of samples where both the virus and vector present.

b = the number of samples where the virus present but the vector was absent.

c = the number of samples where the vector present but the virus was absent.

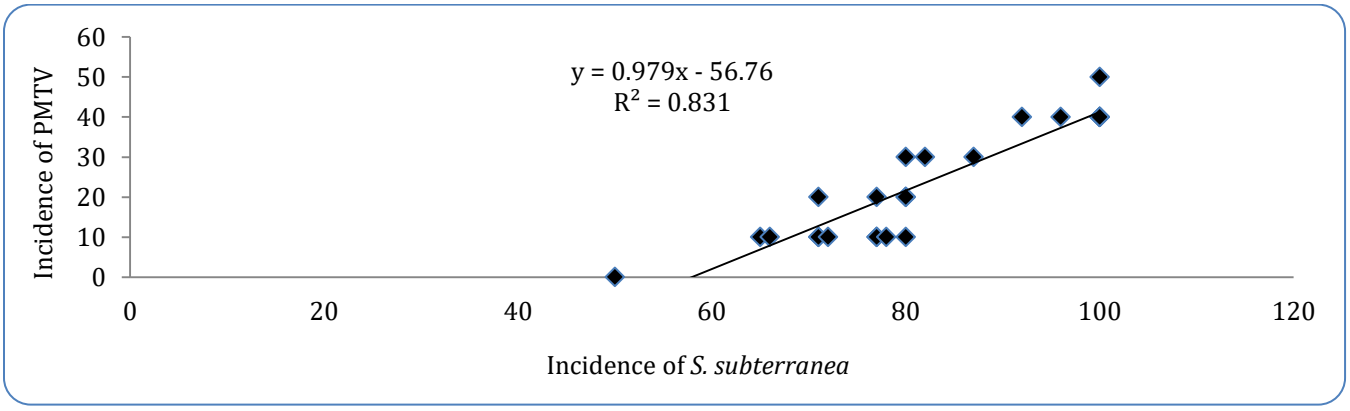

Figure 1: Regression analysis of PMTV on *S. subterranea* on the basis of external symptoms.

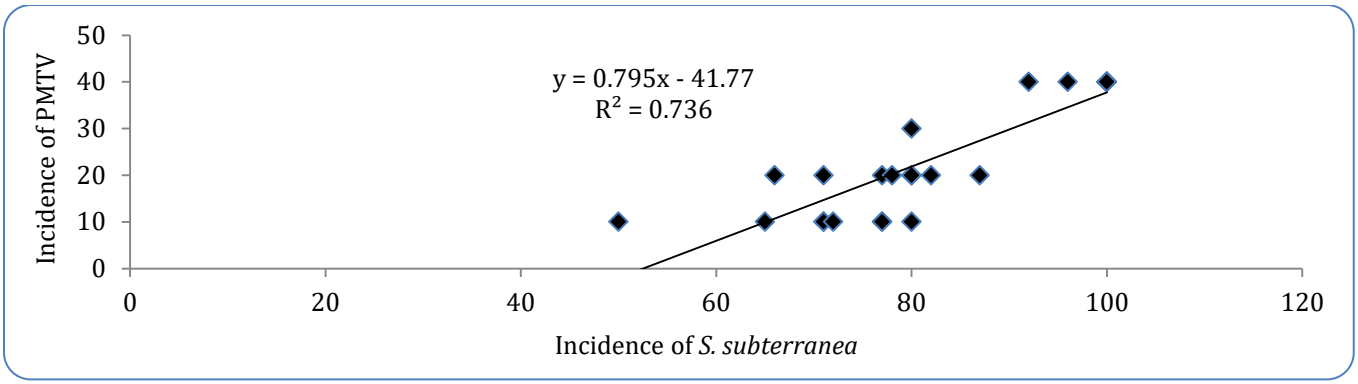

Figure 2: Regression analysis of PMTV on *S. subterranea* on the basis of internal symptoms.

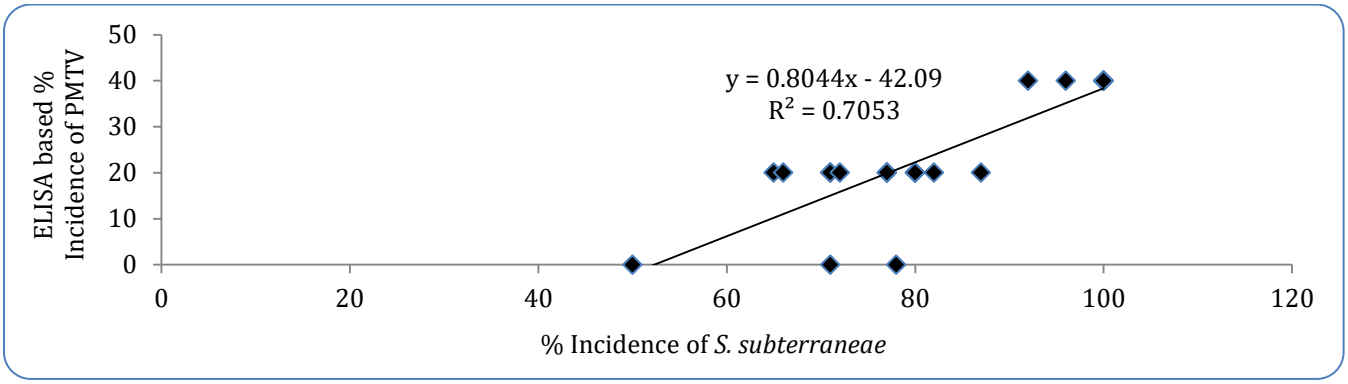

Figure 3: Regression analysis of PMTV on *S. subterranea* based on ELISA detection.

Assessment of association between TRV and its vector

Positive linear regression relationships were computed between TRV and its vector. The 1.76% increase in TRV incidence result of 1% population increase of *Trichodorus minor, Paratrichodorus* during the growing season (P-value= 0.000) a significant association (R^2 0.83) between vector and TRV (Figure 4). The regression analysis between TRV incidence and nematode vector based on internal symptoms on potato tubers is shown in (Figure 5). The value of R^2 = 0.45, which is lower than the threshold value (R^2=0.5). Such interaction is said to be statistically significant (P-value=0.000). ELISA-based regression analysis on incidence of TRV and incidence of its vector show positive relationship (P-value= 0.000). With 1% increase in incidence of nematode vector in the field conditions, the incidence of TRV is also increased by 1.71% (Figure 6).

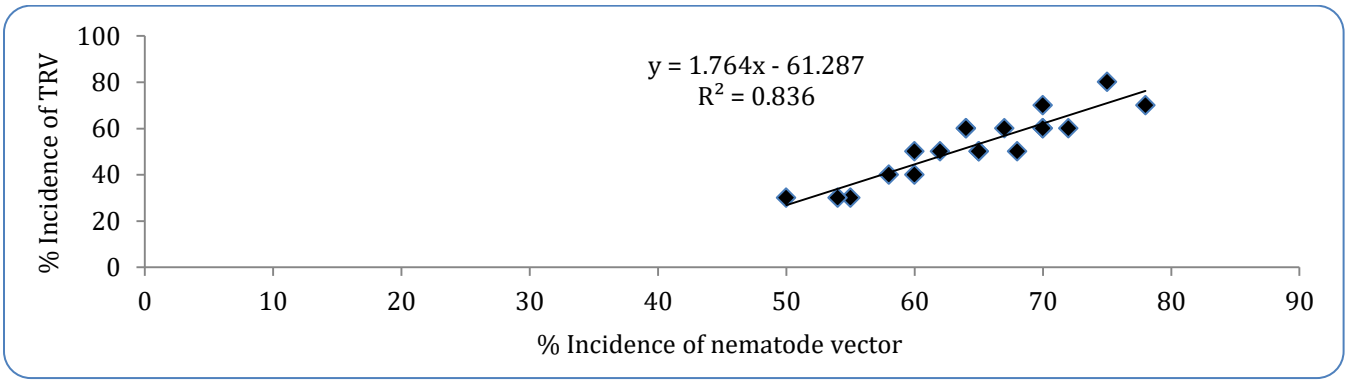

Figure 4: Regression analysis of TRV on nematode vector based on external symptoms.

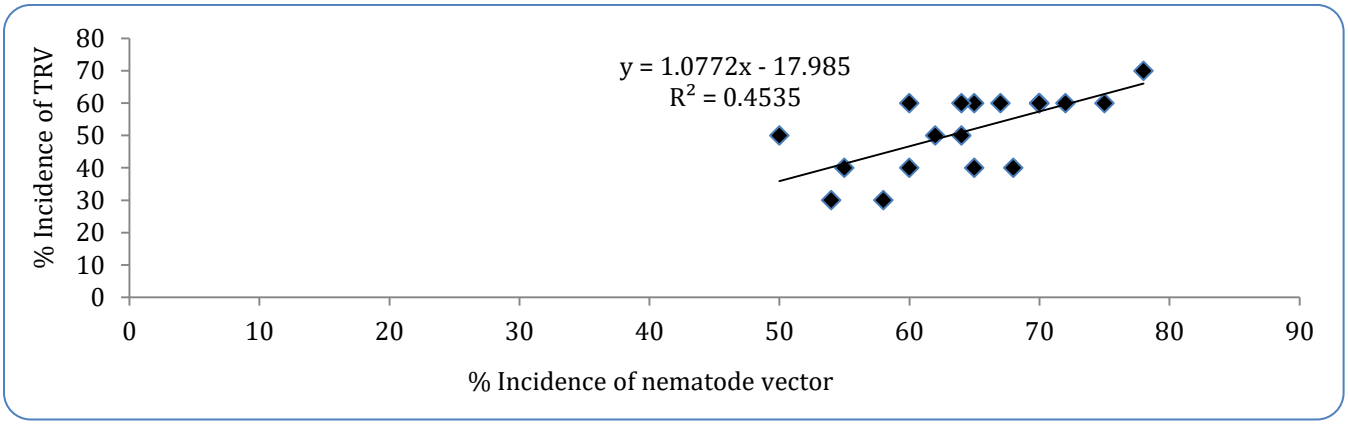

Figure 5: Regression analysis of TRV on nematode vector based on internal symptoms.

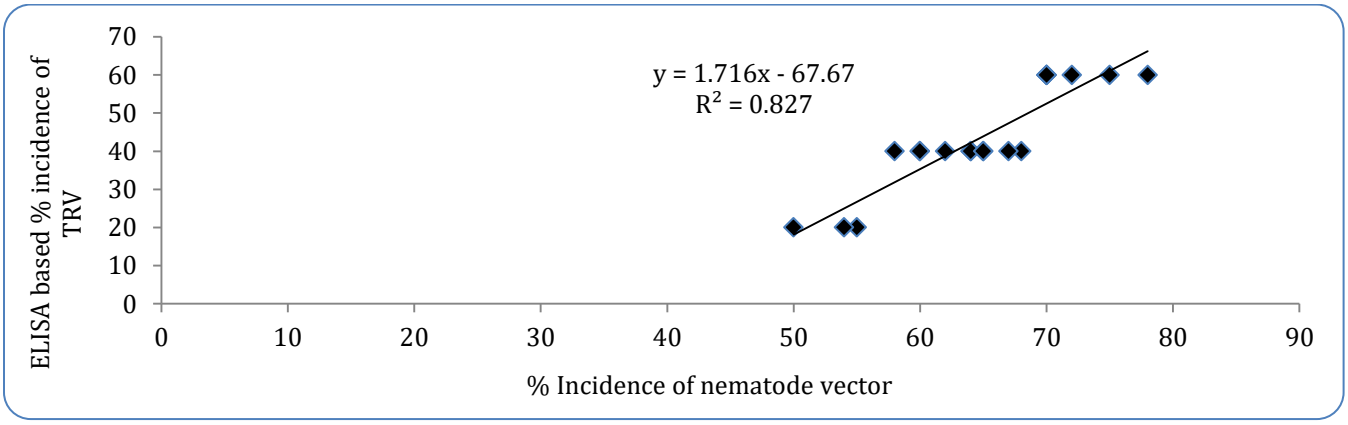

Figure 6: Regression analysis of TRV on nematode vector based on ELISA detection.

Interaction estimation of PMTV severity and *S. subterranean*

The correlation between incidence of PMTV and disease severity was statistically significant as P-value is 0.037 (Table 7). The bivariate Pearson correlation between incidence of PMTV and *S. subterranea* incidence was found associated with disease severity under field conditions are summarized (Table7). *S. subterranea* positively enhanced the disease severity (r=0.20), but it is not much influenced.

Impact of nematode vector on infection of TRV

Incidence of nematode vector and TRV disease severity were also found to be positive correlated (r=0.18) though statistically insignificant, an indication of poor positive correlation. Slight positive association was determined (r=0.053) between nematode vector presence of TRV which was detected by ELISA method and was non-significant (P-value=0.81) (Table 8). Correlation analysis estimates revealed severity of TRV and nematode vector increase with the increase of its incidence but inconsistent way.

DISCUSSION

Soil-borne viruses (PMTV and TRV) and their vectors were prevalent in Malakand and Hazara divisions of Khyber Pakhtunkhwa and selected locations in Azad Kashmir. The incidence of both viruses and their vectors was found higher in Malakand and Hazara division than Azad Kashmir. The variability in the incidence amongst the localities and in individual field may be due to the soil type, climate, and altitude difference. The average incidence of *S. subterranea* was recorded quite higher than the incidence of PMTV in Malakand, Hazara and selected areas of Azad Kashmir, respectively. The higher incidence of the fungal virus vector (*S. subterranea*) in this region is already reported (Ahmad *et al.*, 1996; Iftikhar *et al.*, 2007).

Table 7: Correlation between PMTV and *S. subterranea* incidence and disease severity.

`	Pearson correlation (r)	P-value	No. of observations
PMTV (incidence and severity)	0.427	0.037	24
Spongospora subterranea (incidence and severity)	0.206	0.333	24

*. Correlation is significant at the 0.05 level.

Table 8: Correlation between TRV and nematode vector incidence and disease severity.

Pathogen	Pearson correlation (r)	P-value	No. of observations
TRV (incidence and severity)	0.053	0.87	24
Nematode vector (incidence and severity)	0.184	0.452	19

High humidity and moisture level in field facilitate the germination of zoospores in acquisition and transmission of PMTV in field with the help of its fungal vectors (Teakle, 1988). It increases virus infested seeds and increased field contaminations with local seed multiplications for a long time in the same fields. 3-4 crops per year further enhance incidence without disease-free certified potato seeds. Both temperature and humidity affect the association between the virus and the vector, in PMTV and S. subterranea or TRV and its nematode vector. The temperature and humidity are changing with altitude in the North-western seed potato producing regions, and same trend were found around the world (Delfosse *et al.*, 2002). The other reason for the highest incidence of both virus and the vector must be the multiplication of local seeds for a long time. The flow of virus-free certified seed is limited in the system and import of germplasm without proper quarantine regulations. Growing 3-4 potato crops in a year enhances the virus-vector inoculum in the region.

The interaction and association of viruses (PMTV; TRV) and their vectors was calculated by Jaccard Similarity Index and linear regression (Montero-Astúa *et al.*, 2008). Regression analysis showed significant relationship (P-value=0.000) between viruses (PMTV; TRV) and their vectors. The corresponding R^2 indicates positive association between viruses and their vector. Statistically, significant regression means that with the increase in the incidence of the vectors in the field the incidence of viruses also increases. Pearson correlation was applied to determine the relationship between the incidence and severity of viruses (PMTV and TRV) and also for their vectors (*S. subterranea* and *Trichodorus minor*, *Paratrichodorus spp.*). The relevant Pearson correlation indicates positive correlation indicated an increase in incidence of viruses and their vectors, also increasing the disease severity (Chaudhry, 1988).

In general, the present study indicated that where higher incidence of the soil-borne vectors (both plasmodiophorid and nematodes) was found, the incidence of the soil-borne viruses (PMTV and TRV) were high. However, magnitude of associations between virus and vectors was low as pervious findings (Nielsen and Nicolaisen, 2003; Tenorio *et al.*, 2006; Montero-Astúa *et al.*, 2008). As in case of PMTV most of the potato cultivars are susceptible to both the virus and the vector (Arif *et al.*, 2013; Iftikhar *et al.*, 2007). The Association of TRV and its nematode vector is still needed to be explored. Stringent need for quarantine implementation and surveillance to avoid the disease (De Boer, 2000; Merz *et al.*, 2005) and disease-free seed for crop multiplication are imperative to avoid the diseases. The study helps in understanding relationship status of Plasmodiophorid, nematode-transmitted viruses and their vectors and nature of association. Exploration regarding candidate genes against PMTV and TRC cannot be ignored for long. Natural resistance genes in potatoes are not yet documented against PMTV in potato cultivars worldwide (Santala *et al.*, 2010; Valkonen, 2007). Evaluation of potato germplasm from various parts of the world is needed to be identified for durable plant disease management.

CONCLUSION

PMTV and TRV are found in the potato crop grown in KPK AJK, and a positive correlation exists between disease incidences of virus vectors. Vectors were prevalent in the North-western region of Pakistan with significant associations between viruses and their vectors with low magnitude. A positive correlation was found between incidence and severity of soil-borne viruses and their vectors.

ACKNOWLEDGMENTS

This work was partially funded by Higher Education Commission, Islamabad, under National Research Program for Universities (NRPU) through Grant No. 20-1182/R & D/08.

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CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

AUTHORS CONTRIBUTIONS

All the authors contributed equally to this work.