ONLINE ISSN: 3007-3197 | PRINT ISSN: 3007-3189

www.amresearchreview.com

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 4 (2025)

SURVEY AND MANAGEMENT OF VIRAL DISEASES IN STRAWBERRY (*Fragaria ananassa* Duch.) CROPS IN DISTRICT SWAT

Madieha Ambreen[1], Fawad Khan*[2], Farkhanda Manzoor[3], Imtiaz Ali Khan[4], Samina Yasmin[5]

Article Details

Keywords: Viral Diseases, Strawberry, District Swat

[1]Madieha ambreen
Assistant Professor, School of Biology (Botany Section), Minhaj Lahore, Pakistan

[2]Fawad Khan (Corresponding Author)
Medical Entomologist, Health department Khyber Pakhtunkhwa Entomology Department, Faculty of Chemical and Life Sciences, Abdul Wali Khan University Mardan, Khyber Pakhtunkhwa, Pakistan.
Email: medicalentomologist94@gmail.com

[3]Farkhanda Manzoor
Dean of Scientific Research and Development, Minhaj University, Lahore, Pakistan

[4]Imtiaz Ali Khan
Professor, Department of Entomology, The University of Agriculture, Peshawar, Pakistan

[5]Samina Yasmin
Lecturer, Department of Zoology, Hazara University, Mansehra, Khyber Pakhtunkhwa, Pakistan

ABSTRACT

Strawberry (*Fragaria ananassa Duch*) is among the most important berry fruits that belong to the family *Rosacea* and the genus *Fragaria*. A number of viruses are known to infect strawberries, strawberry strawberry reducing their yield drastically. Field survey was conducted to figure out the status of viral diseases in strawberry crop of district Swat, Khyber Pakhtunkhwa, Pakistan. The survey result revealed the incidence and prevalence of viruses infecting strawberries in district Swat during the growing season 2021-22. Symptoms like mosaic, necrosis, chlorosis, crinkling, and reddish discolouration of leaves and petioles were observed in all the strawberry growing areas surveyed. DAS-ELISA results also confirmed the presence of *Strawberry latent ring spot virus* (SLRSV), *Strawberry mild yellow edge virus* (SMYEV) and *Tomato ring spot virus* (ToRSV) in strawberries of district Swat. The highest average disease incidence (14.7 %) was recorded for SLRSV, followed by SMYEV and ToRSV (14 % each). In a single field, the highest SLRSV incidence was as high as 20.0 % in the village of Sherpalam tehsil Matta.

In contrast, the lowest disease incidence was recorded in the village of Baghderai tehsil Khwaza Khela (11.7 %). Maximum disease incidence of SMYEV in any single area was recorded at 16.7 % also at the village Sherpalam tehsil Matta, followed by 15 % infection at village Baghderai tehsil Khwaza khela, the lowest disease incidence of 11.7 % was recorded at village Duresh khela tehsil Matta. The highest recorded ToRSV disease incidence in a single individual area was 16.7 % again at village Sherpalam tehsil Matta, whereas the lowest incidence was recorded at 11.7 % at Khwaza khela, district Swat. In the viral disease management experiment, among the three insecticides used to control the aphid vector, ButlerbutlerbutlerButler was comparatively better in controlling the aphid vector and, hence, the virus transmission. The order of the effectiveness of different insecticides in the management of viruses was Butler, Actara, and Bolton, with the highest number of aphids (24.51) and high disease incidence (25.67 %) recorded in control where no insecticide was used. From the data, it is concluded that the insecticide butler gives the best result against aphids, followed by Actara and Bolton.

Introduction

Strawberry (*Fragaria ananassa Duch*.) is an important soft fruit crop, which is a member of the family *Rosaceae* and the genus *Fragaria*. The strawberry fruit is categorized as aggregate fruit (Green, 1971). It has a pleasant taste and aroma and is believed to be a rich source of potassium, fibre, phenols, sugars and natural antioxidants (glutathione, flavonoids, and vitamins) (Anwar *et al.,* 2015). The combination of different nutrients (Basu *et al.*, 2013) makes it an excellent fruit for human health (Schwab *et al.*, 2009). It is a good source of vitamin C and has great dietary value. It contains 89 % water, 0.07 % protein, 0.5 % fats, and 8.4 % carbohydrates. A 100 g strawberry serving contains 35 Kcal energy, 89.97 g water, 0.11 g total lipids, 0.43 g protein, 2.1 g dietary fibre, 9.13 g carbohydrate, 4.5 g total sugar, 0.75 mg Fe, 16 mg Ca, 11 mg magnesium (Mg), 148 mg K, 13 mg P, 0.049 mg Cu, 0.022 mg riboflavin, 0.29 mg vitamin E, 17 mcg folate, 0.462 mg niacin, and 27 mcg β-carotene (Basu *et al.*, 2010; NNDSS, 2009). This fruit is not only used in fresh form but is also preserved in the form of Jams, Jellies, and squashes that can be used during off-season (Galletta and Bringhurst, 1995). The top producer of StrawberryStrawberry in the world is the United States of America, contributing 30 % of the share in the world production (Boriss *et al.,*2012). The production of StrawberryStrawberry is increasing day by day, with a total production exceeding 4 million tonnes words wide (Sharma et al., 2018).

Strawberries contain a maximum amount of antioxidants, which give protection in response to damaging free radicals, and they have been linked with lesser incidence and death rates of heart disease and cancer and with high health benefits (Ames et al., 1993; Wang et al., 1996). This crop needs low temperatures, so it is grown in tropical and sub-tropical regions (Asad, 1997). In Pakistan, it is harvested from April to May. In Khyber-Pakhtunkhwa, the districts of Mardan and Charsadda, and in Punjab Lahore and Islamabad are the leading StrawberryStrawberry producing areas from where it is distributed to other parts of the country. The maturity period for strawberry fruit is shorter and comprises 30-40 days. It is consumed right after picking due to its perishable nature and needs proper care during transportation to different markets (Amin, 1996).

In Pakistan, strawberries have recently been introduced and have a low yield per acre compared to other countries of the world. This low yield is due to a number of reasons, including biotic and abiotic factors. Biotic factors include bacterial infections (angular leaf spot, bacterial wilt), fungal diseases (Alternaria fruit rot, anthracnose fruit rot, black leaf spot, Cercospora leaf spot, leaf blotch, leaf rust) and viral diseases (strawberry ringspot virus, strawberry mild yellow edge virus etc.). Among the biotic stresses, viral diseases of StrawberryStrawberry have significant importance.

A number of viruses, diseases with virus-like symptoms and mollicutes have been reported to affect strawberries (Martin & Tzanetakis, 2006). It may cause a 30 per cent yield reduction that may go as high as 80 per cent, especially when the infection is a complex of different viruses (Thompson and Jelkmann, 2003; Martin & Tzanetakis, 2006). It has been reported that under field conditions, seven aphids transmitted, five nematode transmitted and two whitefly-transmitted viruses infect strawberries, such as *StrawberryStrawberry vein binding virus* (SVBV), *Strawberry mild yellow edge virus* (SMYEV), *Tomato ring spot virus* (ToRSV) and *Strawberry latent ring spot virus* (SLRSV) (Sharma *et al.*, 2018). Recently, a novel virus has emerged with the name Strawberry pallidosis virus (SPaV) and is considered a serious virus infecting StrawberryStrawberry. These viruses produce a number of symptoms in infected plants; however, the type of symptom varies depending upon the virus, its relative proportions, environmental conditions, strawberry cultivar and the stage at which the infection occurred (Martin & Tzanetakis, 2006). Loss of plant vigour and total yield are adversely affected no matter whether the infection is by a single or combination of different viruses, causing a huge impact and making the strawberry crop worthless. A complex of different viruses is believed to be a serious threat to StrawberryStrawberry all over the world.

Strawberry latent ring spot virus (SLRSV) was first identified many years ago (Lister, 1964). It is a member of the family *Secoviridae* and the genus *Nepovirus*. It is spherical, non-enveloped, having a diameter of about 25-30 nm, and has a segmented linear genome, which is bipartite, measuring around 5.6-7 kb in length. The virion is also called rhubarb virus 5 or the Aesculus line pattern. This virus is transmitted through mechanical inoculation, as well as through two genera of nematodes, *Longidorus* and *Xiphinema* and is also believed to be transmitted through seeds and pollens. SLRSVs have been reported worldwide, especially in Europe (Tang et al., 2013).

The *Strawberry mild yellow edge virus* (SMYEV), for the first time, was reported in California in 1922 with an infection rate greater than 30 % (Converse et al., 1987). It belongs to the genus Potexvirus of the Alphaflexiviridae family. The virus is isometric, non-enveloped, with a diameter of 23-28 nm. The angular profile is without discrete Capsomere arrangements (Jelkmann, 1991). SMYEV is transmitted semi-persistently by aphid species in the Chaetosiphon genus, mainly *Chaetosiphon fragaefolii, C. thomasi, C. fragaefolii, and C. thomasi* Jacobi (Martin & Tzanetakis, 2006). This virus possibly needs an additional virus for transmission through vectors, mostly luteovirus (Jelkmann, 1991).

Strawberry vein banding virus (SVBV) is described as the earliest DNA virus in the crop and was first recorded in the 1950s. Genome has double-stranded DNA and is transmitted by grafting technique or by aphids through a semi-persistent manner (Frazier & Converse1980). It is worldwide in distribution on cultivated strawberry crops (Petrzik et al., 1998). It belongs to the genus Caulimovirus (Petrzik et al., 1998). It is transmitted by at least 3 Chaetosiphon species of aphids, *C. fragaefolii, C. Thomas, and C. Jacobi*, in a semi-persistent manner. Viruses produce symptoms like leaf curling and vein banding as well as leaf necrosis on *F. vesca* and *F.virginiana* (Frazier & Morris, 1987). In a single infection, this virus does not cause severe symptoms on commercial cultivars but produces severe symptoms only if plants are infected with multiple strawberry viruses.

Tomato ringspot virus (ToRSV) taxonomically comes under a genus of Nepovirus. The virus is icosahedral, having a diameter of about 28 nm, sedimenting as three components during sedimentation and exists in many perennial crops (Brunt et al., 1996), affecting 285 species of plant in 159 genera of 55 botanical families (Edward Son & Christie, 1997). The virus is transmitted through sap inoculation, but seed transmission has also been reported in several crops. The virus is also transmitted by vegetative propagation as well as through pollens. Transmission through species of nematode genera *Xiphinema* and *Longidorus* is also reported. The characteristic symptom produced by ToRSV is ring spotting on leaves.

Up to 80 per cent yield losses, especially in case of multiple infections, are reported in strawberries (Thompson & Jelkmann, 2003; Martin & Tzanetakis, 2006). Multiple infections with various viruses, owing to their synergistic interaction (Thompson *et al.,* 2003), pose a severe threat to the cultivation of the crop and certified saplings all over the world. Martin *et al.* (2013) reported eleven viruses infecting strawberries throughout the USA and Canada.

As per our knowledge, no work has been done on the detection, incidence, identification and loss assessment of viruses in strawberry crops of Pakistan in general and particularly in Swat. Therefore, the current research project is planned to achieve the objective as follows.

Materials and Methods

Survey of strawberry growing areas and sample collection.

During the strawberry growing season 2021-22, five locations in districts Swat were surveyed. In each location, two fields were selected. Thirty samples were randomly collected from each field, irrespective of whether the collected samples were from plants having the symptoms or not, by moving in an X pattern through the strawberry field and picking leaf samples after a fixed number of steps. The collected sample was packed into an envelope, labelled and brought to the University of Agriculture Peshawar for sample preservation at 4^{o}C at the Plant Virology Laboratory for further sample processing for disease incidence and characterization.

Serological Assay for virus detection

Plant samples collected during the survey were tested for the presence of SLRSV, SMYEV, and ToRSV using DAS-ELISA (Double antibody sandwich enzyme-linked immunosorbent Assay) (Clark & Adam 1977).

Procedure for DAS-ELISA

The coating, conjugate antibodies, and buffers were used at the recommended dilution according to the manufacturer's procedure (Adgia, USA).

Diluted virus-specific antibodies were used to coat all the wells of the ELISA plates (100 µl) through dilution in coating buffer (1:2000v/v). The plates were incubated at 37^{o}C for 2 hours. Then, they were washed three times using a washing buffer.

Leaf samples were properly crushed in extraction buffer in plastic bags at the rate of 1:5 w/v, and 100 µl of the sap was added per well. After aliquoting, all the crushed samples were incubated at 4^{o}C overnight in the refrigerator. The next day, all the plates were washed three times using a washing buffer.

After washing the plates, 100 µl of the diluted conjugate antibodies at the rate of 1:2000 v/v were aliquoted into each well of the plate, followed by incubation at 37 C for 3 hr.

Following the incubation, washing was done thrice again. The substrate p nitro phenyl phosphate diluted @1 mg/ml in substrate buffer was aliquoted in each well. The plates were incubated in the dark at room temperature.

To confirm the presence of a virus, the plates were scored visually after 30 minutes, 60 minutes, and 120 minutes of incubation for the development of yellow colour.

Viral disease incidence

The per cent incidence of the infecting virus was figured out employing the following formula as reported by Teng and James (2002)

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 4 (2025)

$$\text{Disease incidence (\%)} = \frac{Number\ of\ infected\ plants}{Total\ number\ of\ observed\ plant} \times 100$$

Identification and extraction of Nematode vector

The nematodes were extracted from each set of 20g soil samples using a tray-and-sieve method and counted under a stereo microscope. Based on their morphological traits, they were then identified under a compound microscope (40 X magnification) using a published identification key (Loof & Luc,1990).

Management of vector-borne viruses using insecticides

A field experiment was conducted using RCBD (randomized complete block design) in district Swat. In each treatment, the size of the plot was 6 × 4 m and was replicated four times. There were four treatments, including the control. Three different insecticides (Table 3.2) were sprayed at the recommended doses on the appearance of aphid vectors in the field. In control plots, fresh tap water was sprayed on the crop. The field was kept as recommended using routine horticultural practices with rows of untreated plants between the treatments to nullify the effect of other treatments. Virus presence was confirmed through DAS ELISA assay. The efficacy of each treatment was determined by taking and analyzing data on yield and yield parameters given as under.

- Plant height
- Single fruit weight
- Yield per plant

The population of aphids was counted on three leaves (Top, middle, bottom) from three plants, selected randomly in each treatment. The number of aphids was recorded at a biweekly interval.

Experimental AnalysisAnalysisAnalysis and Layout

The recorded data were analyzed by using Analysisthe AnalysisAnalysis of variance (ANOVA) applicable to RCBD. To test the difference between treatment means Least significant difference (LSD) test was carried out at a 5 % significance level ($P \leq 0.05$) (Steel & Torrie,1980)

Table 3.1 Field layout of an experiment for the efficacy of different insecticides for control of aphids.

Block 1	Block 2	Block 3	Block 4
T1	T3	T4	T1
T3	T2	T3	T2
T4	T4	T1	T4
T2	T1	T2	T3

T1= Actara(Thiamethoxam)
T2=Bolton (Chlorpyriphos+ Gamma cyhalothrin)
T3=Butler (Chlorpyrifos)
T4=Control

Results

Symptoms expression in strawberry crop

During the field survey of strawberry crops, it was noticed that a variety of symptoms were quite prevalent in all the areas surveyed. Strawberry plants exhibited viral disease symptoms such as mottling, mosaic, chlorosis, crinkling, cupped leaflets, necrosis, and stunted plant growth at all the surveyed localities. Discoloration of petioles and leaves having reddish margins was commonly observed in strawberry fields of district Swat. Necrosis and chlorosis were the most prevalent symptoms in all the strawberry fields of the surveyed areas.

Incidence of prevalent viruses as determined by DAS-ELISA

The presence and confirmation of the viruses in collected samples were done using DAS ELISA assay to record virus incidence in strawberry crops of district Swat. The incidence of SMYEV, SLRSV and ToRSV was revealed in all the fields surveyed. However, none of the collected samples tested positive for SVBV, as revealed by polymerase chain reaction results. SLRSV was the prevalent virus recorded in all the strawberry fields of district Swat. The maximum average disease incidence recorded for SLRSV was 14.7 % (Table 4.1), followed by mean incidence as high as 14.0 % each for SMYEV and ToRSV. In an individual field, the maximum recorded incidence of SLRSV was 20.0 % in the Sherpalam area, followed by 15 % at Duresh Khela and 13.3 % each at Takhtaband and Khwaza Khela. In contrast, the lowest incidence of SLRSV was 11.7 % at Baghderai (Table 4.1). The highest disease incidence of SMYEV was recorded at 16.7 %, recorded at Sherpalam, district Swat, followed by 15 % incidence at Baghderai, 13.3 % each at Takhtaband and Khwaza Khela. In comparison, the lowest incidence was 11.7 % at Duresh Khela, district Swat. The second most frequently occurring virus was SMYEV infecting strawberry plants in all the fields of surveyed areas (Table 4.1).

The results of the sample analysis to record the disease incidence of ToRSV show that the highest disease incidence, as revealed by serological assay, was 16.7 % at Sherpalam, followed by 15 % at Duresh Khela and 13.3 % each at Takhtaband and Baghderai. In comparison, the lowest disease incidence (11.7 %) was recorded at Khwaza Khela, district Swat.

Table 1: Table showing the frequency of major viruses infecting StrawberryStrawberry as revealed by DAS-ELISA in district Swat

	SLRSV			SMYEV		T_oRSV		
Areas Surveyed	Samples tested	Samples positive	% incidence	Samples positive	% incidence	Samples positive	% incidence	Mean incidence
Takhtaband	60	8	13.3	8	13.3	8	13.3	
Sherpalam	60	12	20.0	10	16.7	10	16.7	
Khwaza khela	60	8	13.3	8	13.3	7	11.7	
Durushkhela	60	9	15	7	11.7	9	15	
Baghderai	60	7	11.7	9	15	8	13.3	
Mean incidence			14.7		14		14	14.23

The serological assay (DAS ELISA) results revealed that SLRSV was the most frequently infecting virus in district Swat, followed by SMYEV and ToRSV. The sieve extraction method

for soil samples confirmed the presence of the Dagger nematode, a nematode that vectors strawberry viruses like ToRSV and SLRSV.
Nematode identification was made on the basis of its morphological features having a long tube-like body (4-6 mm long), which is J-shaped relaxed (Fig 4.16). Both male and female *xiphinema* sp were present in soil samples taken from district Swat. The body cuticle with characteristic spears (Odontostyle). There was a guiding ring at the odontostyle base before the odontophore junction. A characteristic feature was the flanged odontophore with visible posterior tripartite flanges. In female nematodes, the valval slit was near half of the body length from the mouth side. They also have amphidelphic symmetrical genitals. The uterus was close to the vagina and with a developed objector.

Management of vector-borne viruses using insecticides
The experiment was conducted to show the efficacy of different insecticides at different intervals. After 14 days (Table 4.2), the maximum numbers of aphids were controlled by ButlerbutlerbutlerButler (4.57), followed by Actara (8.75), while the maximum number of aphids (10.70) was recorded in the control block. After 28 days, again, the maximum number of aphids was controlled by ButlerbutlerButlerButler (3.50), followed by Actara (5.42), while the maximum number of aphids (16.27) was recorded in control. After 42 days, the highest number of aphids was controlled by ButlerbutlerbutlerButler (2.95), followed by Actara (3.27) and Bolton (4.45), while the maximum number of aphids (24.51) was recorded in control. Minimum infection (10.22 %) was recorded in plots where ButlerbutlerbutlerButler was sprayed to control the aphids. This was followed by plots treated with Actara (13.89 %) and Bolton (19.89 %). Maximum infection (25.67 %) was recorded in plots where no treatment was applied to control the aphids.

Table 2: Table showing aphid population in the control experiment.

Treatment	After 14 days	After 28 days	After 42 days
Actara	8.75 ABC	5.42 DE	3.27 DE
Bolton	9.32 AB	5.67 CDE	4.45 DE
Butler	4.57 DE	3.50 DE	2.95 E
Control	10.70 A	16.27 B	24.51 A

Table 4.3 Yield parameters, plant height, single fruit weight and yield recorded for different insecticides.

Treatments	Per cent Infection	Plant height (cm)	Single fruit weight(gm)	Yield (gm/m^2)
Actara	13.89C	26.78 B	14.33 B	376.22B
Bolton	19.89B	26.11 B	12.56 B	359.44C
Butler	10.22D	30.78 A	17.00 A	413.78A

Control	25.67A	23.44 C	12.44 C	359.22C
LSD 0.05	0.6927	0.8079	0.6195	2.5804

Effects of insecticides on yield and plant height

Yield

Significant differences (P<0.0065) were seen among different treatments. The maximum yield (413.78 g/m2) was observed in plots where the ButlerbutlerbutlerButler was sprayed, followed by Actara (376.22 g/m2) and Bolton (359.44 g/m2) treated plots. In comparison, the minimum yield (359.22 g/m2) was recorded for control plots (Table 4.3).

Weight of a Single strawberry fruit (g)

The mean values of the weight of individual strawberry fruit are presented in Table 4.3. The maximum single fruit weight (17.00 g) was observed for the plot where the ButlerbutlerbutlerButler was sprayed, followed by plots treated with Actara (14.33 g) and Bolton (12.56 g). In comparison, the minimum single fruit weight (12.44 g) was observed in control (Table 4.3).

Plant height (cm)

Huge differences (P<0.003) were also seen among different treatments in the case of plant height. The maximum plant height (30.78 cm) was recorded for ButlerbutlerbutlerButler, followed by Actara (26.78 cm) and Bolton (26.11 cm), while the minimum (23.44 cm) plant height was observed for control (Table 4.3).

Discussion

Viral diseases cause both qualitative and quantitative yield losses in the infected crops. The strawberry crop has also been infected by numerous viruses under natural field conditions, such as SLRSV (Schmelzer,1969), SMYEV (Martin & Converse,1985), SVBV (Martin & Tzanetakis,2013), and ToRSV (Converse,1981) and many others. In district Swat, virus-like symptoms in strawberry crops were commonly observed in all the fields in the area surveyed. Strawberry leaves exhibited a range of viral symptoms such as mottling, mosaic, chlorosis, leaflet cupping, necrosis red, dish discolouration at the margin of the tender leaves and petioles, etc. These virus-like symptoms have also been reported in a number of research finding in strawberry crops (Jelkmann,1991; Rott *et al*.,1995) across the world in strawberry growing areas. It has been reported that various cultivars of strawberry exhibit symptoms with different intensities of mottling, decline, and patchy and reddish leaves that eventually lead to yield losses of varying scales (Belli *et al*., 1980). Plants show a range of foliar symptoms resulting in yield fluctuation under field conditions Specially serious losses have been reported when infection is as a result of mixed infection by two or more than two viruses.

Although Strawberry Strawberry is a somewhat newly introduced crop in Pakistan, the farmers in the Strawberry Strawberry growing areas complained about the successive increases in the incidence of viral diseases over the past couple of years. The results of the present study also confirmed the presence of viruses in all the surveyed areas infecting strawberry crops such as SLRSV, SMYEV, and ToRSV. These results based on symptomatic evaluation were further confirmed through serological assay, and it was revealed that SLRSV was the dominant virus having high disease incidence in all the fields as compared to other viruses. This high incidence of SLRSV may be due to the wide host range of the virus as reported previously (Murant, 1974), infecting not only strawberry crop but also other fruits crops, including blackberries, plums, black currants, grapes, cherries, and many other dicot plants (EPPO/CABI, 1996; Schmelzer, 1969). Both SLRSV as well as ToRSV are transmitted by *Xiphinema* sp, commonly known as dagger nematode (Lister, 1964; Lamberti et al.,1986), which was also detected in soils of district Swat. During the present survey, it was revealed during the farmer's interviews and by the research officers at ARI, Swat, that ever since its introduction in Pakistan, only one variety, Chandler, is predominantly grown in all the runner production as well as commercial production areas. Strawberries are propagated through runners, a vegetative method of propagation, and in case the infection occurs in mother plants, it is not possible to grow a healthy crop next year. Selection of runners is very crucial, which is made very difficult, keeping in view the trend of strawberry viruses' infection in commercial cultivars of StrawberryStrawberry and the production of symptoms in the infected plants. In the case of a single infection, one can hardly find any symptoms on the infected plant (Thomson & Jelkmann, 2003; Martin & Tzanetakis, 2006). Symptoms get pronounced in case of multiple infections with more than one virus (Thompson & Jelkmann, 2003; Martin & Tzanetakis, 2006). Hence, while selecting the runners for the next crop, it is impossible to know whether it is virus-infected or virus-free unless and until it is tested using serological or molecular assay. This is one of the major reasons for the prevalence of viruses in strawberry crops all over the globe.

SVBV could not reported in any location in district Swat. Not a single band was seen in the gel when amplification was done using specific primers in a polymerase chain reaction (the primer set was from the CP encoding region of SVBV) in any strawberry leaf tested and sampled from strawberry fields of district Swat. It is also reported that among the aphid-transmitted viruses of strawberries (Frazier, 1955), SVBV is the least common virus infecting strawberries and is irregularly reported with very low occurrence across the world (Tzanetakis *et al.,* 2004).

Being a cash crop, the farmers apply pesticides indiscriminately to control various aphid vectors. This excessive and indiscriminate application of pesticides might control the insect vectors. However, not soil-borne nematodes, vectors of SLRSV and ToRSV, and this is one of the reasons for the predominant presence of these two viruses. Mild infection in propagative material, along with the presence of vectors, makes the situation worse. Different weeds act as alternative hosts for these viruses and act as a primary inoculum for all these viruses, which cause a high incidence (Pala *et al*., 2017). Also, StrawberryStrawberry is propagated through runner's asexual method of propagation, and infection of parent plants will result in a high incidence of different viruses. It is worth mentioning that many vegetables are grown in district Swat, which may also guarantee the presence of the virus as well as its vector during the growing period.

DAS ELISA results showed that SMYEV was the second dominant virus in the surveyed localities. The host range of the virus is broad and infects different crops of the family *Rosacea* and *Rosacea* (Jelkmann,1991). Aphids transmit SMYEVAphids transmit SMYEV in a semi-persistent manner that belongs to *the Chaetosiphon* genus primarily, *C. Jacobi, C. ThomasThomasThomasThomas C. fragaefolii,* which which which makes it economically an important virus causing serious yield losses in various crops, including StrawberryStrawberry as well (Martin & Tzanetakis,2006). The aphid vectors have been observed in all the crop surveyed localities, although the densities of vector populations were very low, which might be the indiscriminate application of insecticides. The semi-persistent nature of virus transmission coupled with the occurrence of aphids in the field, though very low in number, results in the successful transmission of the virus. However, as mentioned earlier, excessive use of insecticides and a semi-persistent nature of transmission resulted in a low percentage of infection in district Swat compared to other Strawberry strawberry-growingstrawberry-growing areas of the province. Strawberry is propagated through an asexual method (runners). If the parent crop is infected, the probability of inoculum presence in the field during early stages is very common. It might increase with time, even at low vector population densities. Commercial varieties of StrawberryStrawberry do not exhibit any characteristic symptoms in case of infection caused by any virus alone (Martin & Tzanetakis, 2013), making the runner selection very difficult. The strawberry growers have no information regarding the viruses, their transmission and subsequent spread under field conditions, which is an important reason behind the frequent occurrence of viral diseases in the area. In Pakistan, no work has been done on the prevalence and characterization of viruses infecting strawberries; therefore, the farmers are left with no choice but to grow the crop using uncertified, non-non-tested runners. During the survey, it was observed that the weed population is also very high in the strawberry-growing areas. Among these, many weeds might be the source of primary inoculum acting as the alternate host for viruses infecting strawberries and hence play an important role in inoculum built up.

The insecticides applied to control the semi-persistently viruses are believed to be an efficient method to control viruses, compared to non-persistently transmitted viruses, reducing its incidence in quite an effective manner (McKirdy & Jones, 1996; Perring *et al.*,1999), which is not much effective in case when the virus is transmitted non persistently (Perring *et al*., 1999; Thackeray *et al*., 2002). Our results coincide with the results of Khan and Begum (2000) and Sultana et al. (2009), who have reported that insecticides can effectively control aphid population and hence reduce the incidence of aphid vector diseases. It has been reported by Thompson *et al.* (2003) that under field conditions, transmission of viruses infecting strawberries through vectors (aphids) can effectively be managed by using insecticides. Effective and early management is indispensable for good yield and better healthy crops. It has also been reported that both the infecting virus and vector interaction have evolved in a way that facilitates the easy acquisition of the virus and its subsequent quick spread (Mauck & Bosque-Perez, 2012). The viruses which are already infecting host plants restrict the activation of host defences that facilitate the feeding insect vector and its population build, which might increase the chances of transmission (Abe *et al.*, 2012; Zhang *et al.*, 2012). Infecting viruses also modify the host plants' attractiveness to the insect vector and their infestation for prolonged acquisition and rapid dispersion (Maris *et al.*, 2004; Werner *et al.*, 2009). It has been reported that viruliferous aphids prefer healthy plants (Ingwell *et al.*, 2012; Dara, 2015) and probe them more than the non-viruliferous vectors (Stanfford *et al.*, 2011). Strawberry is a commercial crop and is cultivated in more than 74 countries. In Pakistan, Strawberrystrawberry is a high-income-producing crop and is cultivated on an area of 78 hectares. In Khyber Pakhtunkhwa Swat is considered as the center point of the StrawberryStrawberry where strawberry nursery is produced and is supplied all over Pakistan. The typical yield of strawberry crops is low in our country as compared to other

StrawberryStrawberry producing countries of the world. This low yield is due to several factors. The utilization of uncertified runners, adaptation of poor cultural practices, and lack of awareness about viral infections and their vectors adversely affect the production. Various parasites like fungi, bacteria, nematodes and viruses are responsible for this low yield. Among various biotic factors, viral diseases of StrawberryStrawberry play a great role. A field survey was conducted in the Swat district of Khyber Pakhtunkhwa. Samples were collected from various areas to find the incidence and distribution of major viruses, including SLRSV, SMYEV, SVBV and ToRSV infecting strawberries. The significant symptoms recorded on strawberry plants were yellowing of leaves, necrotic spots, mosaic, and mottling. The samples were processed through serological techniques (DAS-ELISA) for identification of the viruses and to find the disease incidence.

DAS ELISA procedure was utilized for the detection of viruses infecting StrawberryStrawberry. DAS- ELISA result shows that SLRSV was prevailing all over the surveyed areas of district Swat. The highest average disease incidence of SLRSV was recorded (14.7), followed by the average incidence of SMYEV (14) and ToRSV (14). The highest disease incidence of strawberry latent ring spot virus in any single area was as high as 20 % at Sherpalam tehsil Matta. In comparison, the minimum incidence was 11.7 % in the village Takhtaband tehsil Babozai Swat. The mean incidence of these viruses revealed the prevalence of SLRSV, followed by SMYEV and ToRSV. The Average incidence of strawberry mild yellow edge virus was recorded at 14 %.

The highest disease incidence of Strawberry Strawberry mild yellow edge virus in any single area was as high as 16.7 % in the village Sherpalam tehsil Matta. In comparison, the minimum incidence was 11.7 % in the village Duresh Khela tehsil Matta district Swat.

The highest average disease incidence of ToRSV was recorded at 31.26. The highest disease incidence of Tomato ring spot virus in any single area was as high as 16.7 % at village Sherpalam tehsil Matta. In comparison, the minimum incidence was 11.7 % at Khwaza Khela district Swat.

A field experiment was conducted at Mingora Research Institute Swat. The field was prepared in which strawberry runners were transplanted during the season. The field was split into 4 blocks, and each block was further split into four parts. Three different insecticides were sprayed on each block, and one was selected as a control. Insecticides were sprayed at fortnight intervals. The first treatment was given on March 29th 2022. The second treatment was given on April 15th; similarly third treatment was given on April 29th. Data on aphid counting was taken after each interval. After 14 days, the minimum number (4.57) of aphids was counted in the block where the ButlerbutlerbutlerButler was sprayed, followed by Actara (8.75) and Bolton (9.32), while the maximum number of aphids (10.70) was counted for control. After 28 days, the minimum number (3.50) was observed in the block where the ButlerbutlerbutlerButler was sprayed, followed by Actara (5.42) and Bolton (5.67), while the maximum number (16.27) of aphids was counted in control. After 42 days, the minimum number (2.95) was observed in the block where ButlerbutlerbutlerButler was sprayed, followed by Actara (3.27) and Bolton (4.45), while the maximum number (24.51) of aphids was counted in control.

The data was also taken on growth and yield parameters. The maximum plant height (30.78 cm) was observed in the block where ButlerbutlerbutlerButler was sprayed, followed by Actara (26.78 cm) and Bolton (26.11 cm), while the lowest plant height was observed for control. A maximum single fruit weight (17.00 g) was observed in the block where the

ButlerbutlerbutlerButler was sprayed, followed by Actara (14.33 g) and Bolton (12.56 g). In comparison, a minimum (12.44 g) fruit weight was recorded for control. The maximum yield (413.78 g/m^2) was calculated in the block where the ButlerbutlerbutlerButler was sprayed, followed by Actara (376.22 g/m^2) and Bolton (359.44 g/m^2), while the lowest yield (359.22 g/m^2) was recorded for control.

Conclusion

The most frequently infecting viruses recorded in strawberry crops were SLRSV, SMYEV and ToRSV in district Swat.

SLRSV was the most dominant virus in district Swat.

SVBV infecting was not revealed from any of the collected samples as detected through PCR.

Symptoms are mild in a single virus infection. Hence, efficient and regular virus diagnosis is very important to protect commercial fields.

Recommendations

Farmer must regularly screen their runners.

It is better to replace the fields, possibly every year, to avoid transmission of these viruses through soil-borne vectors.

Nematode control may help to manage the virus disease incidence quite efficiently, as both the dominant viruses are transmitted by nematodes.

Roughing out plants exhibiting virus-like symptoms and controlling vectors using pesticides will effectively manage the virus disease incidence by controlling the secondary spread in the field.

Literature Cited

Abe J, A . Miyano, K. Komatsu, A. Kanazawa and Y. Shimamoto (2012) Photoperiod insensitive Japanese soybean landraces differ at two maturity locations. Crop Science. 43: 1300–1304.

Ames, B. N., M. K,Shigenaga, and T.M, Hagen .(1993). Oxidants, antioxidants, and the degenerative diseases of ageing. Proceedings of the National Academy of Sciences, 9 0(17), 7 9 | 5 -7 922.

Amin (1996). Progress and prospects of strawberry production in NWFP. Annual Report Agriculture Research Station North Mingora, Swat. 212:4-7.

Asad (1997). Strawberry production and marketing potentials. Advisory Leaflet of MFVDP.30:1-2. Aslam, M. and S. Rasool. 2012.

Babini A. R., M. Cieslinska R. Karesova and M. Cardoni . (2004). Occurrence and identification of strawberry viruses in five European countries. Acta Horticulturae 656:39-43.

Belli G,A Fortusini and G. Vegetti. (1980). Properties of the strain of *strawberry latent ringspot virus*, associated with a resetting of peach in northern Italy. Acta Phytopatholgica Academiae Scientiarum Hungaricae 15: 113-117.

Bolton A. T . 1974. Effects of three virus diseases and their combinations on fruit yield of strawberries. Canadian Journal of Plant Science 54, 271–275.

Bonsi, C., R. Stouffer, and W. Mountain. 1984. The efficiency of transmission of tomato ringspot virus by *Xiphinema americanum* and *Xiphinema lives* Phyto- pathology 74:626.

Bordynko N. B., Hasiow M. Figlerowicz and H. Pospieszny. (2007). Identification of a new strain of *strawberry latent ringspot virus* isolated from black locust (*Robiniapseudocacia* L.). Journal of Phytopathology 155:738-742

Braun, A. J., J. A. Keplinger. (1973) Seed transmission of *tomato ringspot virus* in raspberry. Plant Disease Reporter 57, 431-432.

Brunt A. A., K. Crabtree M. J Dalwitz ,A. J Gibbs & L. Watson. 1996. Viruses of plants. Descriptions and Lists from the video Database. Cambridge, p. 1484.

Clark, M .F and A.N.Adams.1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. Journal of General virology.34:475-483.

Converse, R. H., R. R Martin, & S. Siegel. 1987. *Strawberry mild yellow edge.* USDA Agricultural Handbook. Virus Disease of Small Fruit: 25–29.

Edwardson J. R., & R. G. Christie. 1997. Viruses infecting peppers and other Solanaceous crops Monograph. University of Florida, Gainesville, Volume. 2., No 18: 337–390.

El-Morsy SL, El-Sheikh MA, El-Razik RAA, Youssef SA and Shalaby AA. 2016. Molecular identification of strawberry latent ringspot virus (SLRSV) in Egypt. Journal of Basic and Environmental Sciences 4:24-33.

EPPO/CABI. 1996. Arabis mosaic nepovirus In: Quarantine pests for Europe. 2nd edition (IM Smith, DG McNamara, RR Scott and M Holderness). CAB International Wallingford, U K.

Faggioli F, L. Ferretti G. Pasquini G and M. Barba. (2002). Detection of *Strawberry Latent Ringspot virus* in leaves of olive trees in Italy using a one-step RT-PCR. Journal of Phytopathology 150:636-639.ds

Frazier N. W., and R. F. Converse. (1980): *Strawberry vein banding virus*. CMI/AAB Description of Plant Viruses No. 219.

Frazier, N. W .(1955). *Strawberry vein banding virus.* Phytopathology 45, 307–312.

Frazier, N. W. (1960). Differential transmission of strains of *strawberry vein banding virus* by four aphid vectors. Plant Disease Reporter. 44:436–437.

Frazier, N. W. and T. J. Morris. (1987). *Strawberry vein banding*, p. 16–20. In: R.H. Converse (ed.). Virus diseases of small fruits. USDA ARS Agriculture Handbook No. 631, Washington, D.C.

Galleta & Bringhurst. 1995. "Small fruit culture and nutritional value". The AV is publishing a West report. 5th edition, USA: 357.

Green, A. 1971. Soft Fruits. In: Hulme, A.C (ed.). The biochemistry of fruits and their products. Academic Press. New York. 2: 375–410.

Harison, B. and C.H Cadman 1959. Role of a dagger nematode *Xiphinema* species outbreaks of plant diseases caused by Arabis mosaic virus. Nature, Lond., 184: 1624–1626.

Ingwell L.L, S.D.Eigenbrode and N.A . Bosque-Perez (2012) Plant viruses alter insect behaviour to enhance their spread. Scientific Reports 2: 578.

Jelkmann W, E. Maiss and R. R Martin. (1991). The nucleotide sequence and genome organization of *strawberry mild yellow edge* associated Potexvirus (SMYEAV). Journal of General Virology 73: 475-479.

Jelkmann W, R. R. Martin D. E. Lesemann H. J. Vetten and F. Skelton. (1990). A new potexvirus associated with strawberry mild yellow edge disease. Journal of General Virology 71: 1251-1258.

Kim J, S. J. Lee SK. Choi J. Kim and W. Jang. (2016). Development of Simple and

Rapid Diagnosis Method for *Strawberry Latent Ringspot Virus* in Plants Using Loop-Mediated Isothermal Amplification Assay. Journal of plant pathology and microbiology 7: 377.

Kitajima, E. W. J. A. Betti, and A. S. Costa. (1973). *Strawberry veinbanding virus*, a member of the *cauliflower mosaic virus* group. Journal General Virology. 20, 117–119.

Kulshrestha S, V. Hallan G. Raikhy R. Ram and A. A. Zaidi. (2004). *Strawberry latent Ringspot virus* infecting roses in India. The American Phytopathological Society 88:86.3-86.3.

Kumar N. A, Narasu M. L, Zehr U. B and Ravi K. S. (2009). Molecular

Characterization of *strawberry latent ringspot virus* (SLRSV) in Egypt. Journal of Basic and Environmental Sciences 4:24-33.

Lamberti, F. and T. Bleve-Zacheo. (1979). Studies on *Xiphinemaamericanum* sensuality with descriptions of fifteen new species. Nematologia Mediterranea 7, 51-106.

Lamberti, F., F.Rosa, S. Landriscina, A. Cianio.1986. Seasonal transmissibility of strawberry latent ring spot virus by *Xiphinema diversicaudatum*. *Nematologia Mediterranea* .14: 173-179.

Lawson R. H. S. S Hearon E. L & Civerolo. 1977. Carnation etched ring virus. CMI/AAB Description of Plant Viruses No. 182.

Lister. R. M. (1964).*Strawberry latent ringspot*: A new nematode-borne virus' Annals of applied biology 54:167 -17 6

Loop, P.A. A., & M. Luc (1990). An updated polytomous key for the identification of species of the genus *Xiphinema* in systematic parasitology. 16;35-66.

Maris P.C, N.N Joosten, R.W Goldbach and D. Peters D(2004). Tomato spotted wilt virus infection improves host suitability for its vector, Frankliniella occidentalis. Phytopathology 94: 706–711.

Martin ,R.R.and I. E. Tzanetakis.2013. High-riskHigh-risk strawberry viruses by region in the United States and Canada; implication for certification, nurseries and fruit production.Plant disease. 97; 1358-1362

Martin R. R L. E. Tzanetakis J. E. Barnes J. E & J. Elmhirst. 2004. First report of *strawberry latent ringspot virus* in StrawberryStrawberry in the United States and Canada. Plant diseases 88:575.

Martin R. R and L. E Tzanetakis. (2006).Characterization and Recent Advances in Detection of Strawberry Viruses. Plant Diseases 90:384-396.

Martin R. R. & R. H. Converse R. H. 1982. *Strawberry mild yellow edge luteovirus*. Acta Horticulturae 129: 75.

Mauck K, N.A Bosque-Perez , S.D.Eigenbrode, C.M.Moraes and M.C.Mescher (2012) Transmission mechanisms shape pathogen effects on host–vector interactions: evidence from plant viruses. Functional Ecology 26: 1162–1175.

Mazyad A. A.,A. A. Kheder A. El-Attar and A. F. Amal. (2014). Characterization of *strawberry latent ringspot virus* (SLRSV) on StrawberryStrawberry in Egypt. Egyptian

Journal of Virology 11:219-235.

McKirdy, S., R.A.C.Jones 1996. Use of imidacloprid and newer generation synthetic pyrethroids to control the spread of barley yellow dwarf luteovirus in cereals. Plant Disease. 80, 895–901.

Morris, T. J. R. H. Mullin, D. E Schlegel, A. Cole, & M. C Alosi. (1980). Isolation of calicivirus from strawberry tissue infected with *strawberry vein banding virus*. Phytopathology 70, 156–160.

Mráz I., K. Petrzik M. ŠÍP and J. Fránová-Honetšlegrová. (1998): Variability in coat protein sequence homology among American and European sources of *strawberry vein banding virus*. Plant Disease 82: 544–556

Pala, F., H. Mennan, A.Demir, A.Ocal, M.Z. Karipcin, M.Pakyurek and M.H.Aydin.2017.Effect on weed control of soil disinfection with steam in strawberry farms. In: 4th international Regional Development conference, at Tunceli, Turkey. Pp.226–237.

Perring, T.M, N.M.Gruenhagen, C.A.Farrar(1999). Management of plant viral diseases through chemical control of insect vectors. Annual. Review. Entomology. 44, 457–481.

Rott, M. E., A.Gilchris , L. Lee and D.Rochon.1995.Nucleotide sequence of tomato ring spot virus RNA 1. Journal of General Virology.76: 465-473.

Scarborough, B. A and S. H. Smith. 1977. Effects of tobacco and tomato ringspot viruses on the reproductive tissues of Pelargonium x hortorum. Phytopathology 67, 292–297.

Schmelzer, K., (1969). Strawberry latent ringspot virus in Euonymous, Acacia and Aesculus. Phytopathology 66, 1–24.

Sharma A, A .Handa ,S. Shylla, S. Kapoor ELISA-based detection of Tobacco Streak Ilarvirus in Strawberry. Paper presented at National Conference on Himalayan Biodiversity and Bioresourse Utilization. Shimla on March 23rd, held at Himachal Pradesh University, Shimla, Himachal Pradesh. (2018).

Shepherd R. J. 1981. Cauliflower mosaic virus. CMI/AAB Description of Plant Viruses No. 243.

Smith, K. M. 1972. A textbook of plant virus diseases (edition 3), pp. 541–544. Longman, London, UK.

Stenger, D. C. R. H. Mullin, and T. J. Morris. (1988). Isolation, molecular cloning, and detection of *strawberry vein banding virus* DNA. Phytopathology 78, 154– 159.

Tang J, L. I. Ward & G. R. G. Clover. 2013. The diversity of *strawberry latent ringspot virus* in New Zealand. Plant Disease 97:662-667

Thackray D.J, R.A.C.Jones, (2002). Forecasting aphid outbreaks and epidemics of barley yellow dwarf virus—a decision support system for a Mediterranean-type climate. In: Proceedings of the 7th International Plant Virus Epidemiology Symposium, Ascherseleben, Ger- many, p. 135.

Thomson.M.L.Carroll, R.A.C Jones, (2003). Selection, biological properties and fitness of resistance-breaking strains of tomato spotted wilt tospovirus in pepper. Annual. Applied. Biology., 142, 235–243.

Tzanetakis, E. Ioannis,D.Joseph, Postman, C.Rose, R.Robert, Martin. A virus between families nucleotide sequence and evolution of strawberry latent ring spot virus (2006) 199–204.

Wang, H., C. A. O, G. and R. L. Prior. (1996). Total antioxidant capacity of fruits. Journal of Agriculture and Food Chemistry. 44, 701-70s.

Werner S, E.Diederichsen , M.Frauen , J.Schondelmaier , C.Jung 2009. Genetic mapping of clubroot resistance genes in oilseed rape. Theoretical and Applied Genetics **116**, 363– 72.

Zhang, F., M.L.Maeder,E.Unger-Wallace, E., D., Dobbs, T.Peterson. 2010. High frequency targeted mutagenesis in Arabidopsis thaliana using zinc finger nucleases. Crop science 107, 12028–12033.