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Short communication

Detection of apple mosaic and chlorotic leaf spot viruses by DAS-ELISA from farmers orchards of Kashmir valley

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ABSTRACT

The prevalence of *Apple mosaic virus* (ApMV) and *Chlorotic leaf spot virus* (ACLSV) was investigated in the major apple growing areas of Kashmir valley. Leaf samples collected from fifty two orchards showed virus like visible symptoms. All samples were analyzed to detect the viruses through double antibody sandwich- Enzyme linked immuno sorbent assay (DAS-ELISA). Samples of about 69% of orchard showed infection for ApMV and 73% for ACLSV. Maximum incidence of both the viruses were observed in apple varieties 'Mollies Delicious', 'Red Delicious' and 'Golden Delicious', 'Starkrimson', 'Maharaji' and in variety 'Jonica', 'Chamoora', 'Hazratbali', 'Red Chief', 'American Apirouge' and 'Ambri', virus infection was absent. The present study indicated the occurrence and distribution of ApMV and ACLSV in Kashmir valley.

Key words: Apple, DAS-ELISA, Kashmir, *apple mosaic virus*, *chlorotic leaf spot virus*.

Apple (*Malus domestica* Borkh.) occupies prime position in area and production among all the temperate fruits growing in three states, *viz*., Jammu and Kashmir (J&K), Himachal Pradesh (HP) and Uttarakhand (UK) of North Western Himalayan region of India. The productivity of apple is 13.1 MT/ha in J&K followed 8.8 MT/ha in HP (Anon, 1). The average productivity of apple in India as low as 10 MT/ha in comparison with developed countries (30-60 tonnes/ha). The crop is prone to be affected by several diseases caused by fungi, bacteria and viral pathogens. Other pathogens can be controlled or eliminated by critical observation and taking preventive measures at appropriate times, whereas for viral disease the control is difficult as viral pathogens appears latent infections without showing any visible symptoms, however, reducing crop vigour along with reduction in yield and quality of produce (Campbell, 3; Verma and Sofi, 7). Among many viral disorders reported in apple, ApMV under the genus Illar virus is the most important viral pathogen (Nemeth, 5). Enzyme linked immuno sorbent assay (ELISA) has been routinely used in the indexing, certification and quarantine programmes of different temperate fruit along with apple in many countries (Nemeth, 5). Further, the economic importance of Apple chlorotic leaf spot (ACLSV), under the genus Trichovirus is largely due to its worldwide distribution and causing diseases The most reliable methods for ACLSV are ELISA using virus specific antibodies (Ulubas and Ertunc, 6). In order to minimize infection and propagate virus free planting material and to characterize the biological reaction of apple varieties in response to ApMV and ACLSV, the survey was under taken during the years 2010-11 and 2011-12 in several apple orchards of Kashmir to detect the viral diseases using serological diagnostic method.

Fifty two farmer's orchards were surveyed to examine the presence of foliar symptoms in apple, if any caused by virus and virus-like organism in Kashmir valley. The apple orchard of different areas of Kashmir valley surveyed were Pattan, Sopore, Baramulla, Shopian, Chadoora-Budgam, Bandipora, Zainapora-Pulwama and Achabal-Anantnag (Table 1). Ninety three leaf samples were collected from particular area. Leaf samples showing discernible symptoms of (ApMV) and (ACLSV) were collected and about 5 g leaf sample was macerated in 5 ml extraction buffer (pH 7.4) in mortar and homogenized. The plant extract was transferred to eppendorf tubes and stored at 4°C overnight and used for ELISA. The ELISA enzyme linked immune-sorbant assay was carried out using Bioreba Kit (Bioreba Ag, Christoph Merain-Ring 7 CH-4153 Reinach BL1 Switzerland) containing both primary antibody and conjugate according the vendor produce. Direct antibody Sandwitch- ELISA (DAS-ELISA) was performed for detection of viral disease.

During the survey, the apple tree showed different kind of symptoms viral disorders caused by ApMV and ACLSV were recorded (Fig. 2). The pale to bright creamy spots on the foliage, the typical symptoms of (ApMV) was observed (Fig. 2A). In summer due to high temperature and hot weather conditions the creamy spots turned to necrotic spots (Fig. 2B). In case of

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Table 1. Detection of virus disease of apple caused by ApMV and ACLSV by ELISA in farmer's orchards in Kashmir valley conditions in 2010-2012.

Area	Geographical location	Variety	Per cent detection of	
			ApMV	ACLSV
Parihaspora, Pattan, Baramulla, Kashmir	Altitude – 1598 m; latitude-34.15° North & longitude-74.65° East	Golden Delicious	7.3	4.2
		Mollies Delicious	8.1	6.1
		Starkrimson	4.7	4.7
		Red Delicious	8.3	0.0
		Mean	7.10	3.75
Wanigam, Payeen, Pattan, Baramulla, Kashmir	Altitude – 1678 m; latitude-34.16° North & longitude-74.50° East	Red Delicious	5.7	3.9
		Chamoora	0.0	0.0
		Hazratbali	0.0	0.0
		Mean	1.9	1.3
Pattan, Baramulla	Altitude – 1685 m; latitude-34.16° North & longitude-74.55° East	Golden Delicious	5.8	0.0
		Jonica	0.0	0.0
		Red Chief	0.0	0.0
		Mean	1.93	0.0
Alibagh, Hygam, Sopore, Baramulla	Altitude – 1554 m; latitude-34.28° North & longitude-74.46° East	Red Delicious	3.7	6.9
		Mean	3.7	6.9
Janbazpora, Baramulla	Altitude – 1553 m; latitude-34.21° North & longitude-74.34° East	Red Delicious	6.6	6.1
		Mean	6.6	6.1
Dangarpora, Shopian, Kashmir	Altitude – 1674 m; latitude-34.33° North & longitude-74.41° East	Red Delicious	6.1	7.1
		Golden Delicious	5.0	5.0
		Mean	5.55	6.05
Gogji Pather, Chadoora, Budgam, Kashmir	Altitude – 1566 m; latitude-34.01° North & longitude-74.71° East	Red Delicious	2.3	10.4
		Maharaji	2.1	4.2
		American Apirouge	0.0	0.0
		Ambri	0.0	0.0
		Mean	1.10	3.65
Bandipora, Kasmir, J&K	Altitude – 1623 m; latitude-34.42° North & longitude-74.64° East	Red Delicious	2.1	7.0
		Mean	2.1	7.0
Durpora, Zainapora, Pulwama	Altitude – 1652 m; latitude-33.87° North & longitude-74.90° East	Red Delicious	6.4	4.9
		Mean	6.4	4.9
Koloudh, Achabal, Anantnag	Altitude – 1599 m; latitude-33.73° North & longitude-75.16° East	Red Delicious	5.6	5.6
		Mean	5.6	5.6

ACLSV infection, the infected apple trees produces chlrotic blotches showing irregular diffused chlorotic rings (pear ring pattern) (Fig. 2C & D) and line patterns accompanied with reduced leaf size (Fig. 2E) and growth stunting of affected trees. The visual symptoms caused by ApMV and ACLSV were confirmed by immunodiagnostic assay using DAS-ELISA.

Results indicated that out of 52 sampled orchards 69% found positive with ApMV and 73% with ACLSV using ELISA. The maximum number of *ApMV* and *ACLSV* infected trees virus were found in the variety Mollies Delicious followed by varieties Red Delicious, Golden Delicious, Starkrimson and Maharaji. In leaves collected from 37 orchards, Red Delicious orchards of six districts of Kashmir valley tested by ELISA showed positive photometric reaction that varied from 2.1 to 8.3% for *ApMV* (Table 1) with mean infection being 5.2% (Fig. 1). Similarly, infection of *ACLSV* ranges from nil to 10.4% (Table 1) with mean detection being 5.77% (Fig. 1). In Golden Delicious, the incidence of ApMV varied from 5.0 to 7.3% with mean infection 6.03% for *ApMV* and to 5.0% with mean incidence

being 3.07% for *ACLSV*. In Maharaji ('Versified') from two orchards were tested and the average diseases incidence for *ApMV* and *ACLSV* was 2.10 and 4.20%, respectively. Whereas, in 'Mollies Delicious' incidence of *ApMV* and *ACLSV* was 8.1 and 6.1%, respectively. In Starkrimson, both the mosaic diseases detected and it was 4.7%. One sample collected from each variety of Chamoora, Hazratbali (Benoni), Jonica and Red Chief were found to be free *ApMV* and *ACLSV*.

The studies revealed that *ApMV* and *ACLSV* are prevalent and distributed in the apple orchards in Kashmir valley as confirmed by symptoms and serological detection. Recently, virus occurrence in many commercial apple orchards in Himachal Pradesh has been reported (Brakta *et al*., 2). Although nucleic acid based detection of viral diseases are considered to be more reliable, ELISA based detection method is considered more accessible for routine diagnosis of virus diseases in apple (Kundu *et al*., 4). As the latent infection of viral diseases is common in perennial crops and development of visible symptoms in the infected crops depends on different factors like virus strain, crop varieties/ cultivars and environmental conditions. The molecule based advance method

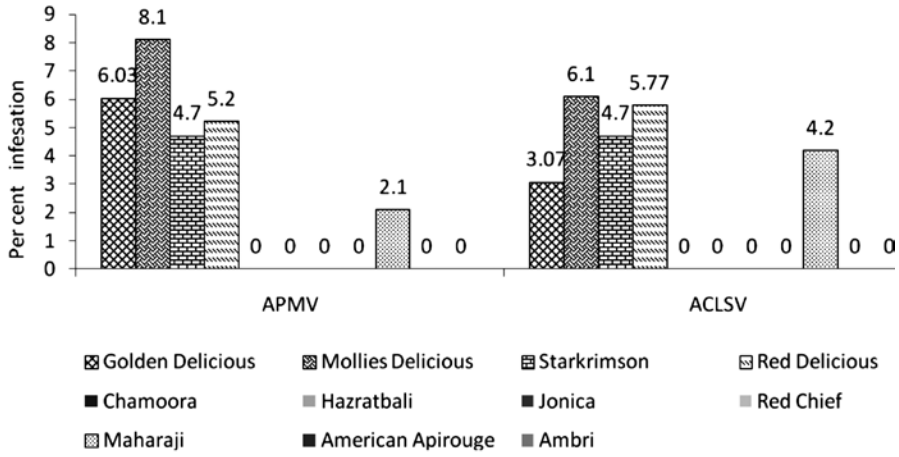

Fig. 1. Percentage infection of *ApMV* and *ACLSV* detected among 11-apple varieties through DAS-ELISA.

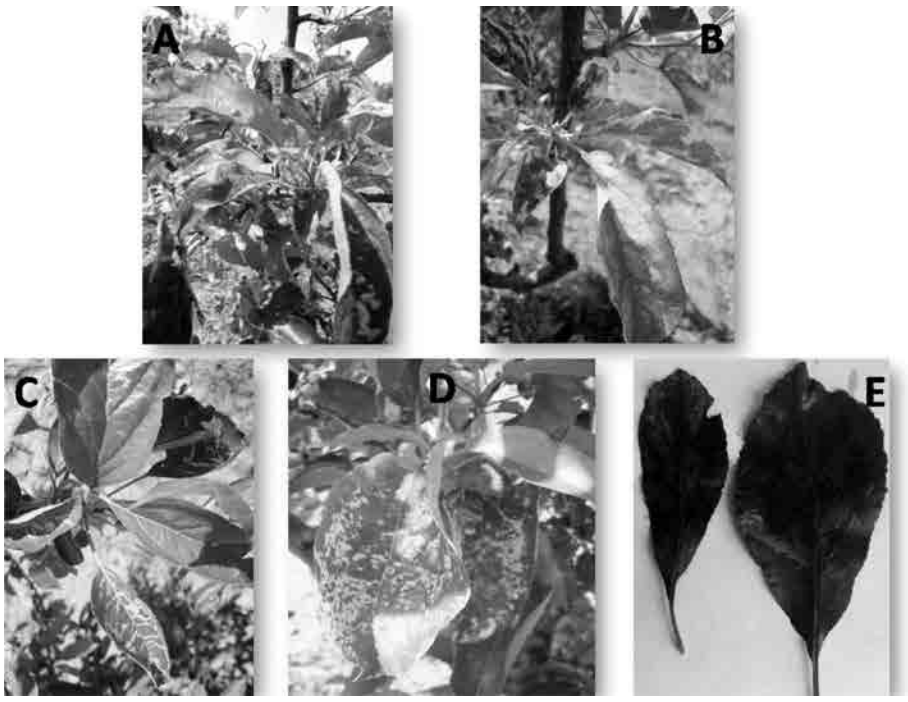

Fig. 2. Symptoms of *Apple mosaic virus* (A&B) and *Apple chlorotic leaf spot virus* (C, D & E).

to detect the virus is essential. Thus, in the present study the serological method exploited showed as important diagnostics tool that will help to develop the management strategy of the virus disease in apple. Further, work is in progress to ascertain the biological response of different cultivars of apple to *ApMV* and *ACLSV* and testing of apple mother stock for production of disease-free planting material. The perennial nature of apple and chronic infection for many years with probable continuous crop-host availability may cause multiple infection of different viruses, therefore certification of planting material and supply of disease free planting material to the apple growers are most essential.

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