Improvement of serological diagnosis of viruses associated with rose mosaic disease affecting Egyptian- rose grown in Taif, Kingdom of Saudi Arabia

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Abstract: Rose mosaic disease is the most common virus disease of roses. It can be caused by one or complex of several viruses including *Prunus Necrotic Ringspot Virus*. Egyptian roses, a great beauty flower with many colors such as red, purple and others. In the recent article, Enzyme-linked immunosorbent assay (ELISA) was used to dignoses of rose mosaic disease in Egyptian rose (*Rosa gallica var. aegyptiaca*) cultivated in Taif, KSA. Twenty five samples exhibited virus- like symptoms of the rose mosaic disease were collected from different locations in Taif governorate, Kingdome of Saudi Arabia;. The serological assay of (DASI-ELISA) indicated that rose mosaic disease in KSA is associated with *Prunus Necrotic Ring Spot Virus* (PNRSV, Genus *Ilarvirus*, Family *Bromoviridae* and *Apple mosaic virus* (ApMV, Genus *Ilarvirus*, Family *Bromoviridae* either as a single or mixed infection. The Polyclonal antibodies (PAbs) specific to PNRSV and ApMV were used to detect PNRSV and ApMV isolates. While**,** all of the 25 tested samples (100 %) gave positive reactions for PNRSV using ELISA with values ranged between 1.370 and 2.308. Only 19 samples representing 76 % were positive for the ApMV with values ranged between 0.198 and 0.256 comparing to the values of 0.061 and 0.071 that detected with the six negative healthy samples. The rose mosaic desieas symptoms appears in some samples, which exhibited mixed infection of the two viruses as chlorotic vein banding, a mosaic pattern and a yellow net pattern. The viral capsid protein of Rose Viruses Associated with Rose Mosaic Disease was estimated to be 25000 Dalton. This is the first report of Egyptian rose viruses in grown in Taif- KSA.

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Key words: Egyptian roses**,** Rose mosaic disease, PNRSV, ApMV, DASI-ELISA, SDS-PAGE.

1. Introduction

The rose is the most popular garden plant in the world, as well as the most important cut flower. There are such a wide variety of roses available that any garden with sufficient sun should be able to grow roses. Rose calls "the Queen of Flowers" because roses have a beautiful shape and smell stay long. Egyptian roses, a great beauty flower with many colors such as Red, purple and other colors. Egyptian roses has a great importance and benefits, it's used as, Kmart and natural antiseptic for skin and gives the skin supple, used in the manufacture of juice and jam, used in the natural landscaping and engineering, used in nurseries and greenhouses harvest crop, used to calm the injured man bouts of anger and annoyance, used in making medical ointments used after radial processors, used in the pharmaceutical industry that improve the flavor of the mouth and the treatment of acne and heal the bruises and wounds, used in the preparation of rose oil and rose water and dried petals, and has a high nutritional value because it contains vitamins (b, c and k).

Virus and virus like diseases of roses have become as common as any of the other rose diseases. All species and varieties of roses are susceptible to one or more virus diseases. However, infection often goes undetected because virus and virus-like symptoms can be mild and easily overlooked (**Horst, 1996**). Rose mosaic disease is the most common virus disease of roses. It can be caused by one or more of a complex of several viruses including *Prunus Necrotic Ringspot Virus* (PNRSV, Genus *Ilarvirus*, Family *Bromoviridae*) (**Fulton, 1970; Moury *et.al.*, 2001; Mansour, 2006**) and *Apple Mosaic Virus* (ApMV, Genus *Ilarvirus*, Family *Bromoviridae*) (**Johnstone *et al.*, 1995; Wong and Horst, 1993; Mansour, 2006**). Infected plants with rose mosaic viruses show different symptoms such as flower distortion; reduced flower production, flower size, stem caliper at the graft union and vigor; early autumn leaf drop; lower bush survival rates;

increased susceptibility to cold injury; and difficulty in establishment after transplanting (**Thomas, 1981, 1982; 1984; Moran *et al*., 1988; Wong *et al*., 1988**).

Symptoms are highly variable and depend on the growing season, temperature, species, cultivar and type of viruses infecting the plant. (**Horst, 1996**).

There is no known natural vector of rose mosaic diseases. These viruses can easily be transmitted from infected mother plants to the progenies, such as roses which are vegetatively propagated by own root cuttings or grafting on rootstock (**Janick, 1986**). Therefore, detection and identification of rose mosaic is necessary, particularly with regard to testing nursery materials and propagation stocks. Advanced diagnostics are also critical to trade among countries, having great potential for importation and quarantine programs. The main objective of this study was to determine the viruses associated with rose mosaic disease in Taif region, KSA using the serological methods.

2.Material and Methodes

The experiments of this study were conducted in the experimental greenhouse at the Biotechnology and Genetic Engineering Center, Taif University, KSA.

Rose sampling:

Egyptian- rose (*Rosa gallica var. aegyptiaca*) plants were surveyed in different Rose farms in Taif, KSA and symbiotic plants were transferred to the experimental greenhouses at Taif University, KSA during the growing season. A Set of 25 leaf samples were collected and 1- 5 gm of mature and young leaves from each sample were stored at 4^{o}C.

ELISA detection:

The method of detection is an Enzyme-linked immunosorbent assay (ELISA) based on
Double antibody sandwich indirect (DASI) by using Polyclonal antibodies. The antigen is trapped by the Fragment F (ab) 2 and revealed by the whole IgG. Signal develops by alkaline phosphatase reaction with p-nitrophenyl phosphate; as described by **Edwars and Cooper (1985)** to detect PNRSV and **Imed *et al.* (1997)** to detect ApMV. Polyclonal antibodies (PAbs) were purchased from Agritest S.r.l., Valanzano, Italy. DASI ELISA results were taken as mean absorbance value of three replicates per sample. Positive and negative controls were supplied with the kit.

Determination of viral protein molecular weight:

Using the polyacrylamide gel electrophoresis (SDS-PAGE), plant preparation of viral protein was denatured by heating in the presence of sample buffer **(Lammli, 1970) and (Hill and Shepherd, 1972)**. The mixture was then boiled in water bath for 5 min and was immediately put in ice before loading on the gel. The denatured gels were prepared as 12% running gel and 4.5% stacking gel. The gels were prepared form monomer solution of 30% acrylamide and 0.8% Bis-acrylamide. Ammonium persulphate and TEMED were used as initiators for cross- linking and polymerization.

Infected Egyptian- rose (*Rosa gallica var. aegyptiaca*) samples which exhibited virus-like symptoms of the rose mosaic disease, from plant sap were prepared separately at ratio 1: 10 in 6 X sample buffer (appendix) and boiled for 5 min in water bath and clarified by centrifugation. Aliquots of 15 µl were applied per slot with low or mid standard protein markers run for 2 hr at 80 volts and a further 90 min at 120 volts. The gel was stained with Commassie Brilliant blue R 250. At the end of the run, when the dye reaches the bottom, the sandwiches were disassembled and the gel was put into staining solution. The gel was gently shaken for overnight at room temperature, then removed from the stain and washed once with water and put in destaining solution for one hr. The gel was transferred to a second container filled with destining solution and shaken for another hour. At this stage, the protein band could be visualized by naked eyes and the data could be recorded by taking a photograph.

3.Resultes and Discution

Rose mosaic disease is the most common virus disease of roses. It can be caused by one or more of a complex of several viruses including *Prunus Necrotic Ringspot Virus* (PNRSV, Genus *Ilarvirus*, Family *Bromoviridae*) (**Fulton, 1970; Moury *et.al*., 2001; Mansour, 2006**) and *Apple Mosaic Virus* (ApMV, Genus *Ilarvirus*, Family *Bromoviridae*) (**Johnstone *et al*., 1995; Wong and Horst, 1993**; **Mansour, 2006**).

ELISA detection:

The 25 rose (*Rosa gallica var. aegyptiaca*) samples which exhibited virus- like symptoms of the rose mosaic disease were tested against PAbs specific for PNRSV and ApMV from Agritest S.r.l., Valanzano, Italy using ELISA. The results are explained in **table 1** and **figure 1**. It was shown that a number of 25 out of the 25 tested samples, represented 100%, gave positive reactions with the PNRSV antibodies with values ranged between 1.370 and 2.308. The reactions with the ApMV antibodies resulted in positive results with19 out of 25 (76 %) tested samples (**Table 2 and figure 2)** with values ranged between 0.198 and 0.256 compared to values ranged between 0.061 and 0.071 of the six negative

healthy samples. The survey suggested that rose mosaic disease is associated with PNRSV and ApMV either as a single or mixed infection. The results of ELISA tests clearly showed that PNRSV was the dominant virus on roses since about 100 % of infected samples were found to be PNRSV-infected either in single or mixed infection. ApMV occupied the second position with 76 %. These results are in agreement with previous reports (**Cambra *et al.*, 1989; Casper, 1973; Fulton, 1970; Moury *et al.*, 2001**). ELISA was widely used for the detection of PNRSV in tissues collected early in the vegetation period in young leaves or in newly formed buds (**Thresh *et al.*, 1977; Barbara *et al.*, 1978, 1979; Barbara, 1988; Thomas, 1980; Mink and Aichele, 1984 a, b; Torrance and Dolby, 1984**).
ApMV was routinely detected by ELISA (**Clark *et al.*, 1976; Voller *et al.*, 1976; Thresh *et al.*, 1977; Barbara *et al.*, 1979; Korpraditskul *et al.*, 1979; Hardcastle and Gotlieb, 1980; Torrance and Dolby, 1984**). ELISA detection can be done throughout the growing season in individual samples of young leaves or twigs with newly formed buds (**Torrance and Dolby, 1984**).

Virus source:

According ELISA results the mixed infection samples of the two viruses were used as a source of virus infection. The highly concentrated +ve-ELISA sample (No.12); Egyptian- rose (*Rosa gallica var. aegyptiaca*) was showing rose mosaic symptoms appears as chlorotic vein banding, a mosaic pattern, and a yellow net pattern as shown in **Figure (3)**.

Symptoms on Egyptian- rose (*Rosa gallica var. aegyptiaca*) were similar to those on Rose trees infected with rose mosaic virus according to (**Horst, 1996; Hagan and Mullen, 2000**) whish reported that Rose mosaic virus has a wide range of symptoms including ring spots, wavy lines, chlorotic vein banding, an oak leaf pattern, a mosaic pattern, and a yellow net pattern.

Determination of viral protein molecular weight:

The viral protein of Rose Viruses Associated with Rose Mosaic Disease migrated as a single band from plant viral preparation with a molecular mass of ~ 25 KDa as shown in **Fig. (4)**.

Rose mosaic disease is the most common virus disease of roses. It can be caused by one or more of a complex of several viruses including *Prunus Necrotic Ringspot Virus* (PNRSV, Genus *Ilarvirus*, Family *Bromoviridae*) (**Fulton, 1970; Moury *et.al*., 2001; Mansour, 2006**) and *Apple Mosaic Virus* (ApMV, Genus *Ilarvirus*, Family *Bromoviridae*) (**Johnstone *et al*., 1995; Wong and Horst, 1993; Mansour, 2006**). The viral protein of ApMV& PNRSV preparation migrated as a molecular mass of ~ 25 KDa. The obtained results were in agreement with previous results that obtained by **Gonsalves and Fulton (1977)** whish described the method of SDS-PAGE protein preparation and reported that ApMV& PNRSV capsid contains a single protein species with mol. wt. of about 25000 Dalton**.**

Table (1): DASI- ELISA detection of PNRSV in Roses (*Rosa gallica var. aegyptiaca*) samples using the PAbs specific to PNRSV from Agritest S.r.l., Valanzano, Italy.

Samples #	ELISA detection	
	EV	R
1	1.619	+
2	1.370	+
3	1.973	+
4	1.377	+
5	1.740	+
6	2.308	++
7	1.648	+
8	2.056	+
9	1.487	+
10	1.438	+
11	1.458	+
12	1.701	+
13	1.946	+
14	1.451	+
15	1.436	+
16	1.580	+
17	1.649	+
18	1.811	+
19	1.956	+
20	1.559	+
21	1.639	+
22	1.471	+
23	1.432	+
24	2.104	++
25	1.640	+

Positive control: **1.667;** Negative control: **0.504**
EV: ELISA values; **R**: Result;
+: Positive; -: Negative

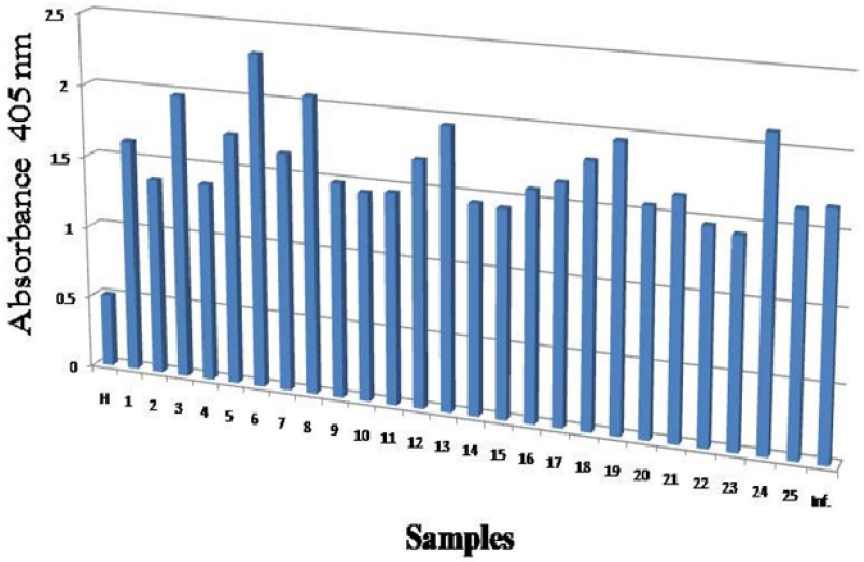

Figure 1: Histogram showing the results of the DAI- ELISA using polyclonal antibodies specific for PNRSV from Agritest S.r.l., Valanzano, Italy, with healthy (H) as a (N.c), Infected as a (P.c.), the samples from 1 to 25 leaf samples of Roses (*Rosa gallica var. aegyptiaca*) seedlings.

Table (2): DASI- ELISA detection of ApMV in Roses (*Rosa gallica var. aegyptiaca*) samples using the PAbs specific to ApMV from Agritest S.r.l., Valanzano, Italy.

Samples #	ELISA detection	
	EV	R
1	0.071	-
2	0.065	-
3	0.070	-
4	0.069	-
5	0.061	-
6	0.062	-
7	0.213	+
8	0.212	+
9	0.204	+
10	0.207	+
11	0.198	+
12	0.251	++
13	0.201	+
14	0.200	+
15	0.212	+
16	0.209	+
17	0.214	+
18	0.256	++
19	0.210	+
20	0.200	+
21	0.213	+
22	0.223	+
23	0.232	++
24	0.215	+
25	0.245	++

Positive control: **0.221; EV**: ELISA values; Negative control: **0.061; R**: Result; **+: Positive -: Negative**

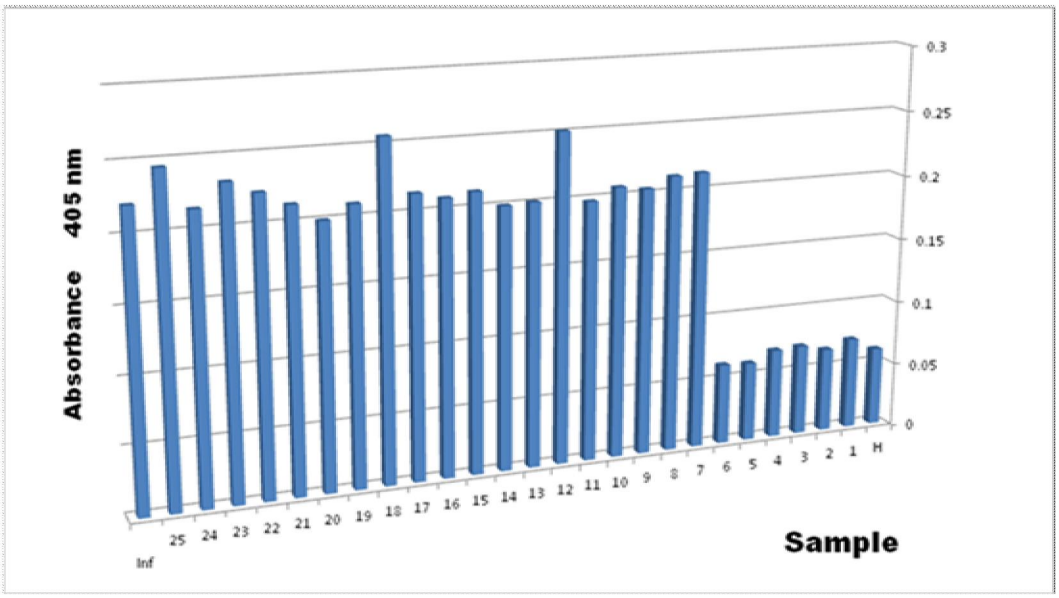

Figure 2: Histogram showing the results of the DASI- ELISA using polyclonal antibodies specific for ApMV from Agritest S.r.l., Valanzano, Italy, with healthy (H) as a (N.c), Infected as a (P.c.), is the samples from 1 to 25 leaf samples of Roses (*Rosa gallica var. aegyptiaca*) seedlings.

Figure 3: Symptoms observed on Rose plants collected greenhouses the experimental farm at Taif University showed Ring spots, wavy lines, chlorotic vein banding, a mosaic pattern, and a yellow net pattern on *Rosa gallica var. aegyptiaca.*

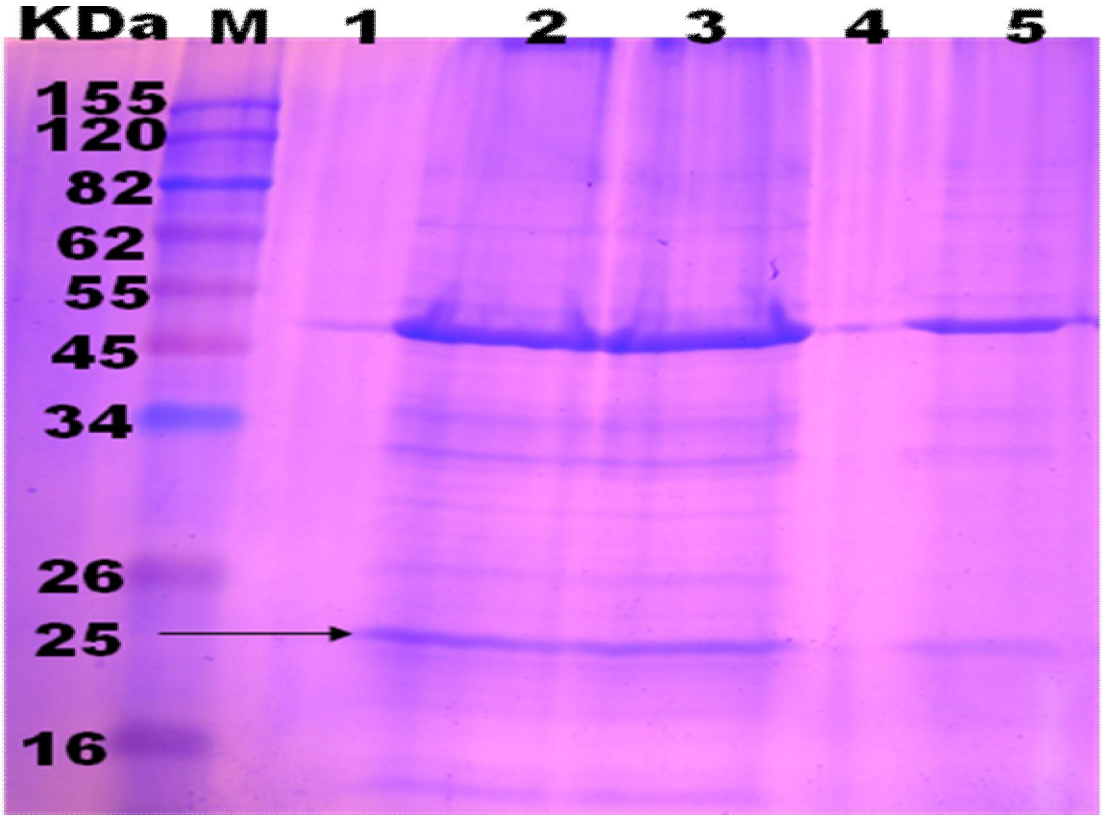

Figure (4): SDS- polyacrylamide gel electrophoresis for plant viral preparation of Rose Viruses Associated with Rose Mosaic Disease showing the viral coat protein band at MW ~ 25 KDa. Lanes 2, 3 and 5 are plant viral preparation. The size marker is the pink plus prestained ladder GeneDirex protein marker.

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Refranses

1. **Barbara, D. J. (1988).** Prunus necrotic ringspot virus (PNRSV). In: ***European Handbook of Plant Diseases*** (Smith I.M. *et al.* Eds.). John Wiley & Sons. pp17- 19. ISBN: 1444314181, 9781444314182.
2. **Barbara, D. J., Clark, M. F., Thresh, J. M., and Casper, R. (1978)**. Rapid detection and serotyping of Prunus necrotic ringspot virus in perennial crops by enzyme-linked immunosorbent assay. ***Ann. Appl. Biol***. 90: 395-399. DOI: 10.1111/j.1744-7348.
3. **Cambra, M., Martinez-Torres, J. L., Benaches, M. J., Camarasa, E. and Gorris, M. T. (1989).** Incidence of Rose Viruses in Spain. ***Acta Horticulturae*****,** 246: 309-312. http://www.actahort.org/books/246/246_40.htm.
4. **Casper, R. (1973).** Serological Properties of Prunus Necrotic Ringspot and Apple Mosaic Virus Isolates from Rose**.** ***Phytopathology,*** 63, 238-240. DOI: 10.1094/Phyto-63-238.
5. **Clark, M.F., Adams, A. N., Thresh, J. M. and Casper, R. (1976).** The detection of plum pox and other viruses in woody plants by enzyme-linked immunosorbent assay (ELISA). ***Acta Horticulturae,*** 67 51-57. http://www.actahort.org/books/67/67_3.htm.
6. **Edwards, M. L. and Cooper, J. I. (1985).** Plant virus detection using a new form of indirect ELISA. ***J. Virol. Methods***. 11: 309- 319. http://dx.doi.org/10.1016/0166-0934(85)90024-2.
7. **Fulton, R. (1970).** Prunus Necrotic Ringspot Virus. ***Descriptions of Plant Viruses***. Commonwealth Mycological Institute and Association of Applied Biologist, Ferry Lane, Kew, Surrey, England. No. (5). http://dpvweb.net/
8. **Gonsalves, D. and Fulton, R. W. (1977).** Activation of Prunus necrotic ringspot virus and Rose mosaic virus by RNA4 components of some ilarviuses. ***Virology*** 81 (2): 398- 407. http://dx.doi.org/10.1016/0042-6822(77)90155-6.
9. **Hagan, A., and Mullen, J. (2000).** *Diseases of Roses and Their Control*. Alabama Cooperative

Extension System. ***ANR***-0505. http://www.aces.edu/counties.

10. **Hardcastle, T. and Gotlieb, A.R. (1980).** An enzyme-linked immunosorbent assay for the detection of apple mosaic virus yellow birch. ***Canadian Journal of Forest Research,*** 10 (3): 278-283. doi: 10.1139/x80-047.
11. **Hill, J. H., and Shepherd, R. J. (1972).** Molecular weight of plant coat proteins by polyacrylamide gel electrophrophoresis. ***Virology***. **47 (3):** 817- 822. http://dx.doi.org/10.1016/0042-6822(72)90573-9.
12. **Horst, K.R. (1996).** *Compendium of Rose Diseases*. ***APS Press, St. Paul, Minn***. p. 151. ISBN 978-0-89054-355-9.
13. **Imed, A.; Boscia, A.; Salderelli, P.; Digiaro, M. and Savino, V.(1997).** A comparison of apple mosaic virus isolates from prunus trees and production of specific monoclonal antibodies. **OEPP/ EPPO Bulletin** 27 (4): 563- 564. DOI: 10.1111/j.1365-2338.1997.tb00686.x.
14. **Janick, J. (1986).** ***Horticultural Sciences***. Fourth Edition, W.H. Freeman and Company, NewYork. 746 p. ISBN-10: 0716717425 | ISBN-13: 978-0716717423 | Edition:4 th.
15. **Johnstone, G. R., Munro, D., Brown, G. S. and Skotland, C. B. (1995).** Serological Detection, Occurrence and Spread of Ilarviruses in Temperate Fruit Crops, Hops and Roses in Tasmania. ***Acta Horticulturae***, 386, 132-135. http://www.actahort.org/books/386/386_16.htm.
16. **Korpraditskul, P.; Casper, R; and Leseman, D. E. (1979).** Evaluation of short reaction times and some characteristics of the enzyme-conjugation in enzyme-linked immunosorbent assay (ELISA). ***Phytopathologische Zeitschrift,*** 96: 281-285. DOI: 10.1111/j.1439-0434.1979.tb01652.x.
17. **Lommel, S. A.; mccain, A. H. And Morris, T. J. (1982).** Evaluation of indirect enzyme- linked immunosorbent assay for the detection of plant viruses. ***Phytopathology*****, 72: 1018- 1022.** DOI: 10.1094/Phyto-72-1018.
18. **Mansour, A. N. (2006).** Identification of Rose viruses associated with rose mosaic disease in Jordan**. Jordan J. of Agri. Sci.** 2 (4): 331-337. journals**.ju.edu.jo/JJAS/article/view/1238.**
19. **Mink, G.I. and Aichele, M.D. (1984a).** Detection of Prunus necrotic ringspot and prune dwarf viruses in Prunus seed and seedlings by enzyme-linked immunosorbent assay. ***Plant Disease***, 68: 378-381. DOI: 10.1094/PD-68-378.
20. **Mink, G.I. and Aichele, M.D. (1984b).** Use of enzyme- linked immunosorbent assay results in efforts to control orchard spread of cherry rugose disease in Washington. ***Plant Disease,*** 68: 207-210. DOI: 10.1094/PD-68-378.
21. **Moran, J. R., Faragher, J. D. and Baker, D. M. (1988).** The Effects of Prunus Necrotic Ringspot Virus on Production and Quality of Rose Flowers. ***Acta Horticulturae***, 234, 429-434. http://www.actahort.org/books/234/234_51.htm.
22. **Moury, B., Cardin, L., Onesto, J. P., Candresse, T. and Poupet, A. (2001).** Survey of Prunus Necrotic Ringspot Virus in Rose and its Variability in Rose and *prunus* spp. ***Phytopathology,*** 91: 84-91. dx.doi.org/10.1094/PHYTO.2001.91.1.84.
23. **Thomas, B. J. (1981).** Studies on Rose Mosaic Disease in field-grown Roses Produced in the United Kingdom. ***Annals of Applied Biology,*** 98 (3): 419- 429. DOI: 10.1111/j.1744-7348.
24. **Thomas, B. J. (1982).** The Effect of Prunus Necrotic Ringspot Virus on Field-grown Roses**.** ***Annals of Applied Biology,*** 100: 129-134. DOI: 10.1111/j.1744-7348.
25. **Thomas, B. J. (1984).** Epidemiology of Three Viruses Infecting the Rose in the United Kingdom. ***Annals of Applied Biology,*** 105 (2): 213-222. DOI: 10.1111/j.1744-7348
26. **Thresh, J. M.; Adams, A. N.; Barbara, D.J. and Clark, M.F. (1977).** The detection of three viruses of hop *(Humulus lupulus)* by enzyme-linked immunosorbent assay. ***Annales of Applied Biology,*** 87 (1) 57–65. DOI: 10.1111/j.1744-7348.
27. **Torrance, L. and Dolby, C.A. (1984).** Sampling conditions for reliable routine detection by enzyme-linked immunosorbent assay of three ilarviruses in fruit trees. ***Annales of Applied Biology,*** 104 (2): 267-276. DOI: 10.1111/j.1744-7348
28. **Voller, A. B; Arlett, A.; Bidwell, D. E.; Clark, D. E. and Adams, A. N. (1976).** The detection of the viruses by enzyme-linked immunosorbent assay (ELISA). ***J. Gen. Virol.,*** 33 (1): 165-167. doi:10.1099/0022-1317-33-1-165.
29. **Wong, S. M. and Horst, R. K. (1993).** Purification and Characterization of an Isolate of Apple Mosaic Virus from Rose in the USA. ***Journal of Phytopathology,*** 139: 33-47. DOI: 10.1111/j.1439-0434.1993.tb01399.x.
30. **Wong, S. M., Horst, R. K. and Langhans, R. W. (1988).** Symptomatology and Occurrence of Apple Mosaic and Prunus Necrotic Ringspot Virus in Rose in New York. ***Acta Horticulturea,*** 234: 437-450. www.actahort.org/books/234/234_53.htm.