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Full Length Research Paper

Incidence and distribution of viruses infecting propagated ornamentals in Northern Iran

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Abstract

During the year 2007-2009 to determine the incidence and distribution of main viral diseases infecting some ornamental plants, samples were collected from ornamental growing glasshouses in Mazandaran and Guilan provinces. Propagated ornamental plants in glasshouses were mostly showing symptoms of systemic necrotic spots and general chlorosis on leaves, growth abnormalities and plant stunting, flower break and flower cup split were also common symptoms. Suspected diseased samples were tested applying routine serological DAS-ELISA, TIBA and Dot-Blot techniques against important diseases of ornamentals, *Tomato spotted wilt virus* (TSWV), *Impatiens necrotic spot virus* (INSV), *Tomato yellow ring virus* (TYRV), *Cucumber mosaic virus* (CMV), *Tomato ring spot virus* (ToRSV) and *Arabis mosaic virus* (ArMV). Selected ELISA positive samples were mechanically back inoculated to specified indicator plants. One the basis of biological and serological reactions the collected ornamental species were identified as a diagnostic natural host for each of the viruses under study. Of the total of 464 ornamental plants (in 23 different families) tested, 224 samples were shown to be infected to the above 6 viruses. On descending order number of infected samples for TSWV were 83 (17.88%), 47 samples for INSV (10.12%), 32 samples for CMV (6.89%), 31 samples for ToRSV (6.68%), 23 samples for TYRV (4.95%), and 8 samples for ArMV (1.72%). Respectively, TSWV and INSV (tospovirus), CMV (cucumovirus) and ToRSV (nepovirus) were the most prevalent viruses infecting ornamental plants in Northern cities of Mazandaran and Guilan province of Iran.

Keywords: Serology, new host, identification, cut flowers, ornamental viruses.

INTRODUCTION

The area under ornamental plants, world cultivation is approximately 230/000 hectors which accounts a market value of nearly 65 billion in $US. Nevertheless the 100/000 hectors of which is glasshouse propagated ornamentals. The EU (European zone) is one of the world's highest densities of flower production per hectare, accounting 10% of the total world area and 44 % of world flower and pot-plant production (Anonymous ,2012; Abasifar, 2003). In recent years, flowers and ornamental plant industry in Iran has gain great developments, 0.1% of the world business market. There are already 8000 production units in the form of 23 flower and plant cooperative societies working with an annual production of 1/186 millions of cut flowers and have a great portion in the economy and employment of human resources (Moghtadaenejad and Abdollvahabi, 2010; Anonymous 2004; Babaei and Azizi 2010). Beside flower production and export difficulties, there are various plant pests and diseases constrain to the industry. Several virus diseases have been reported to infect flower and ornamental plants in Iran which bring losses to quality and quantity of the flower production (Ghotbi *et al.,* 2005, Ghotbi and Shahraeen 2009; Ghotbi and Nazerian 2010; Beikzadeh et al., 2012).

Northern cities of Mazandaran and Guilan are known as an important focal point for ornamental plants production in Iran. Virus diseases are one of the most important problems facing ornamental growers in Iran. Many different plant viruses are reported to infect

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ornamentals worldwide. Some of the most important and damaging viruses infecting ornamentals are reported from the genus tospovirus, nepovirus, potyvirus and cucumoviruses (Loebenstein *et al*., 1995; Suetic *et al*., 1999; Deughtery *et al*., 1997). *Tomato spotted wilt virus* (TSWV), *Impatiens necrotic spot virus* (INSV), *Tomato yellow ring virus* (TYRV) and *Chrysanthemum stem necrosis virus* (CSNV) are the main tospoviruses reported to infect ornamentals worldwide (Loebenstein *et al*., 1995; Peters, 1998). *Cucumber mosaic virus* (CMV-cucumovirus), *Arabis mosaic virus* (ArMV-nepovirus) and *Turnip mosaic virus* (TuMV-potyvirus) has also been reported to be widespread in some ornamentals (Loebenstein *et al*., 1995; Suetic *et al*., 1999; Daughtery *et al*., 1997). In Iran TSWV incidence was first reported on different host plants of petunia, tobacco, cowpea, tomato and cucumber (Bananej *et al*., 1988; Ghotbi and Shahraeen 2008, 2009). Since then there were several report of infection of different species of tospoviruses infecting many different crop plants including ornamentals (Shahraeen *et al*., 2002; Ghotbi *et al*., 2005). TSWV, INSV and a new tospovirus tentatively named Tomato yellow fruit ring virus (TYFRV) were first reported on ornamentals in Markazi and Tehran province (Ghotbi *et al*., 2005; Shahraeen *et al*., 2002; Winter *et al.,* 2005). Damage caused by tospoviruses has been reported in several studies. The severity of infection of up to 100% on has been reported by TSWV and INSV from several *Cinnerea* screen-houses visited in Shiraz province of Iran (Rasoulpor and Izadpanah 2007). Less than 20% of glasshouses grown ornamentals in Unites State has been reported to be infected by TSWV and INSV (Doughtrey *et al*., 1997; German *et al*., 1992). CMV, ArMV and ToRSV are also the most important viruses infecting ornamentals worldwide (Loebenstein *et al*., 1995).

The main nepoviruses reported to infect ornamentals are: *Arabis mosaic virus, Tomato ring spot virus* and *Tobacco ring spot virus* (Loebenstein *et al*., 1995). ArMV is reported from *Arabis hirsute* and *Crocus* spp. in England. This virus is transmitted by a nematode vector (*Xiphinema and Dorylamidae* spp.), mechanical inoculation, grafting and seed (Murant, 1983). In Iran ArMV and ToRSV from *Gladiolus* spp. were reported from ornamentals for the first time (Ghotbi *et al*., 2005; Ghotbi and Shahraeen, 2005). ToRSV reported from America on *Nicotiana tabacum* (Price, 1936) and *Gladiolus* spp. (Leobenstein *et al*., 1995). ToRSV and ArMV transmitted by the vector *Xiphinema* and Dorylamidae spp. ToRSV and ArMV are transmitted by mechanical inoculation, grafting, seeds and pollen. Serological assay, electron microscopy and RT-PCR techniques are reported to be common tests to characterize nepoviruses including ToRSV and TRSV. The aim of this study was to determine the prevalence and percent infection of important viruses occurring on main cultivated/propagated ornamental crops in two region of Iran using routine biological and serological techniques. There was not any comprehensives research on ornamental virus diseases in northern Iran.

MATERIAL AND METHODES

A total of 464 samples were collected in 2006 - 2007 from fields and glasshouses of Guilan and Mazandaran Provinces. These were from 23 different family and 36 plant species (Table 1). Samples, comprised young and fresh leave and stems of each of ornamentals and flowering weeds with various symptom types including leaves and stem deformation, stunting, necrosis of stem and leaves, veinal discoloration, general yellowing of leaves, systemic chlorotic and necrotic spots (Figure 1). Samples for each plant species were selected in random and on the basis of general plant appearance at the time of sampling. In this study, the number and location of the sample species are listed in Table 1. Standard double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (Clark and Adams, 1977) was performed with polyclonal antiserum for ToRSV, TSWV, INSV, CMV, TYRV and ArMV. All serological reagents used against the viruses were from the Bioreba Plant Virus Antiserum collection Company, (Switzerland) including the respective positive controls for each ELISA (TYRV antisera was gifted by laboratory of virology Wageningen, The Netherland). Absorbance at 405 nm was measured with Labsystem multiskan ELISA microplate reader (Denmark). Healthy *N. tabacum* triturated in general extraction buffer was used as negative control. A reaction was considered positive only if the absorbance was more than three times the background mean of negative control. The serological reagents used in ELISA did not reveal any considerable cross reactivity with other virus species of the genus, hence permitted an accurate species identification.

Mechanical transmissions to test plants were done for selected ELISA positives. Samples were prepared by grinding 1 g of leaf triturated in ice cold 0.1 M potassium phosphate buffer, pH 7.0 containing 0.15% of 2-mercaptoethanol. Samples were inoculated to *Nicotiana tabacum, Nicotiana rustica, Petunia hybrida, Chenopodium amaranticiolo* and *Vigna unguiculata* (cv. local Mashad) (Figure1). The test plants were kept in an insect proof greenhouse at a constant temperature of 23-25°C. Symptoms on indicator hosts were recorded every two days for 14 days following inoculation and twice a

Table 1. Natural incidence and distribution of viruses infecting ornamentals in Mazandaran and Guilan provinces.

ornamentales	Total	ToRSV	CMV	TYRV	INSV	TSWV	ArMV	MIX
Ardisia crenata (Myrsinaceae)	7M	-	-	-	-	-	-	-
Orchis pharanopsis (Orchidaceae)	9M	-	-	-	-	2M	-	-
Erica spp. (Ericacea)	6G+14M	-	4G+3M	-	1G+4M	8M+1G	-	2M (INSV+TSWV) 1G (INSV+CMV)
Azali spp. (Ericaceae)	8M	-	-	-	-	-	-	-
Spathiphylum spp. (Araceae)	3G+6M	3M	-	1M	3G+1M	-	-	-
Sterlitzia reginae (Strelitziaceae)	5G+8M	-	2G+2M	-	-	4M+2G	-	1G (TSWV+CMV)
Aglonema schott (Araceae)	2G+10M	-	-	-	-	-	-	-
Anthurium spp. (Araceae)	4G+11M	-	5M	-	1G+2M	2G+2M	-	2M (INSV+TSWV)
Bambusa spp. (Graminaceae)	6M	-	-	-	-	-	-	-
Begunia semperflorus (Beguniaceae)	7M	-	-	-	-	-	-	-
Ficus benjamina (Moraceae)	11M	-	-	3M	-	2M	-	-
Pandanus veitchii(Pandaceae)	4G+5M	-	-	-	-	-	-	-
Scindapsus aureus (Araceae)	5G+7M	-	-	-	2G	1G+3M	-	-
Polysia spp. (Araceae)	5M	-	-	-	-	-	-	-
Bignonia capreolata **(**Bignoniaceae)	4G	-	-	-	-	-	-	-
Zingiber spp. (Zingiberaceae)	14M	-	-	-	3M	-	-	-
Impatiens spp. (Balsaminaceae)	8M	4M	1M	-	3M	-	-	-
Althea spp. (Malvaceae)	10G+5M	2G+1M	-	1G	3G	5G	2M	1G (ToRSV+TSWV)
Chrysanthemum spp. (Compositae)	7G+14M	1G+5M	-	1G	3G+2M	2G+3M	-	-
Dracaena Fragrans(Liliaceae)	7G+4M	-	-	1G+1M	3G	2M+3G	-	-
Diffenbachia amoena (Araceae)	2G+7M	-	-	1G+2M	1M	2M+1G	-	-
Rosa spp. (Rosaceae)	10G+5M	-	-	2G	2G	4G	2G+1M	-
Petris cretrica(Polypodiaceae)	8M	-	-	-	-	1M	-	-
Asplenium scolopendrium (Aspleniaceae)	5G+5M	-	-	-	-	2G+2M	-	-

Table 1 continue

Cupresus sempervirens (Cupressaceae)	5G	-	2G	-	-	1G	-	-
Salvia splendens (Labiatae)	4M	2M	-	-	1M	4M	9M	-
Lilium spp. (Liliaceae)	9M	-	-	-	-	-	-	-
Cycas spp. *(*Cycadaceae)	7M	-	-	-	3M	6M	-	-
spp. (Vitaceae) *Cissus*	8M	-	-	-	2M	-	-	-
Syngonium podophyllum (Araceae)	2G+12M	-	-	2M	1M	-	-	-
Sheflera arboricola (Araliaceae)	7G+13M	2M	-	-	-	-	-	-
hortorum **Pelargonium* (Geraniaceae)	8G+10M	3G+1M	3M	2G	-	1G+4M	1M	-
Pelargonium peltatum (Geraniaceae)	7G	2G+1M	2G+1M	3G	-	1G+4M	-	-
Aspidistra elatior (Liliaceae)	15M	-	-	-	-	-	-	-
Ficus elastica (Moraceae)	11M	-	-	-	2M	-	-	-
Philodendron spp. (Araceae)	6G	-	1M	-	3M	1M	-	-
Pinus spp. (Pinaceae)	7M	-	-	-	-	-	-	-
Camellia sinensis (Theaceae)	10G+2M	-	-	-	-	-	-	-
Codiaeum variegatum (Euphorbiaceae)	4M	-	-	-	1G	1M+4G	-	-
Lilium spp. (Liliaceae)	4M	-	-	-	-	-	-	-
Cordyline spp. (Liliaceae)	5M	-	-	-	-	-	-	-
Dahlia spp. (Compositae)	9G+10M	3G+1M	1G+2M	1G+1M	-	2M	2G	-
Lilium longiflorum (Liliaceae)	8M	-	-	-	-	-	-	-
Beucarnea recurvata (Liliaceae)	5M	-	-	1M	-	-	-	-
Phoenix Canariensis (Palmaceae)	6M	-	-	-	-	-	-	-
Chamaedorea elegans (Palmaceae)	4M	-	-	-	-	-	-	-
Euphorbia pulcherrima (Euphorbiaceae)	7M	-	3M	-	-	-	-	-
Total	464 128G+336 M	31 11G+20M	32 11G+21M	23 12G+11M	47 19G+26M	83 30G+53M	8 4G+4M	-

week for the following 30 days. The indicator plants then tested/rechecked by Elisa to confirm the presence of a particular virus and any symptomless infection.

RESULTS

In this study 464 samples from 46 different propagated

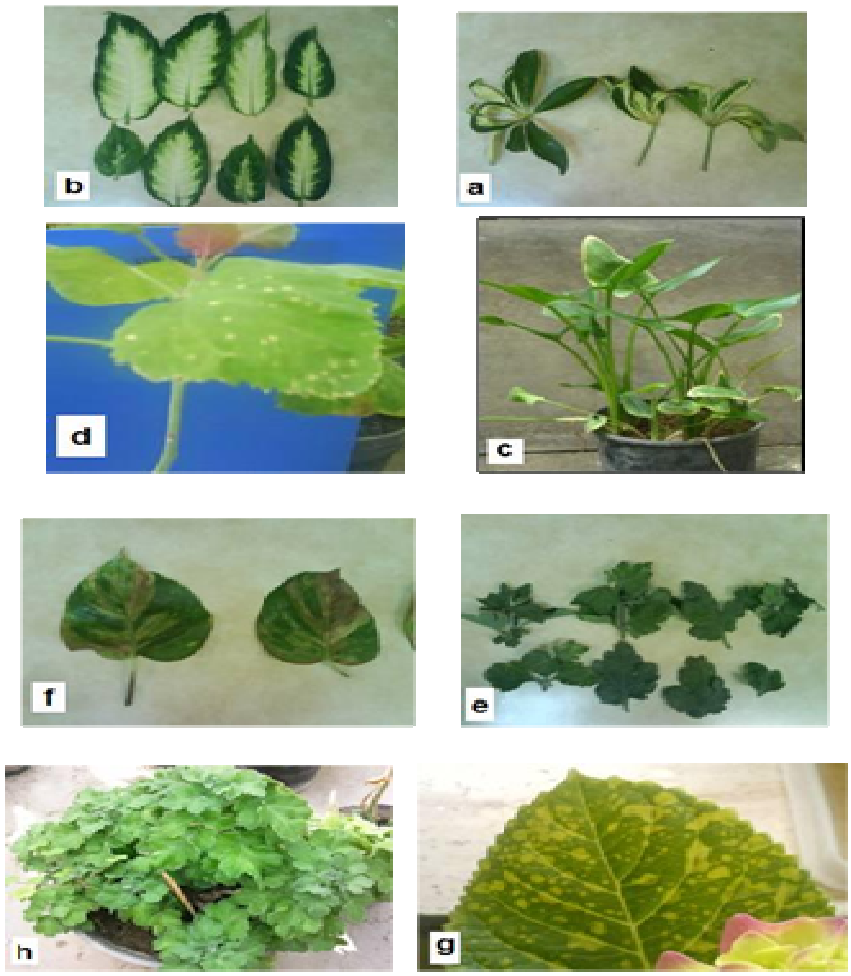
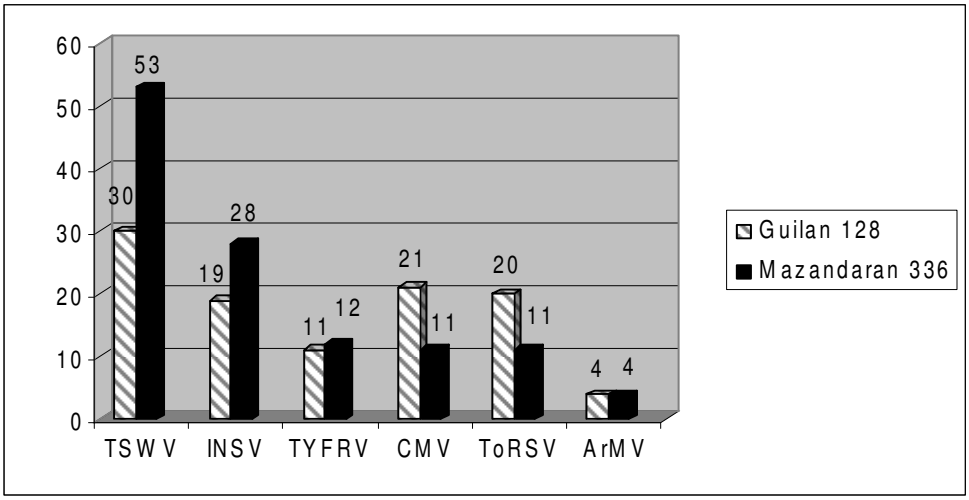


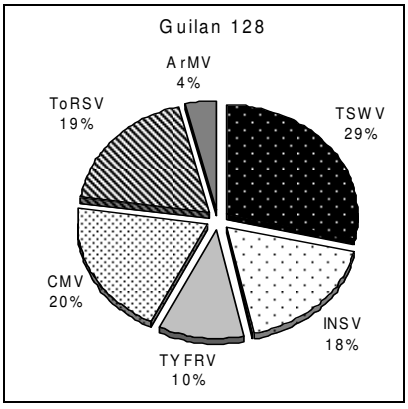
Figure 1.
a- Systemic calico (white centered) like symptoms on *Sheflera arboricola* infected by ToRSV
b- Malformation and little leaf *in Diffenbachia amoena* infected by TSWV
c- Stunting, chlorosis and little leaf in *Philodendron* spp. infected by INSV
d- Local chlorotic pin point spot on *C. amaranticolor* infected by CMV
e- Complexity leaves and chlorotic spots in *Chrysanthemum* spp. (INSV)
f- Mix infection by INSV and TSWV in *Scindapsus aureus*
g- Mosaic and chlorotic spots by CMV in *Euphorbia pulcherrima*
h- Clorotic and stunting in *Chrysanthemum* spp. by TYFRV
i, j- Asymmetry, mosaic and leaf deformation in *Anthurium* spp. Infected by CMV

ornamental species were tested against six worldwide economically important viral diseases infecting ornamentals. On the basis of different biological and serological test reactions TSWV, ToRSV, CMV, TYRV, INSV and ArMV were identified from different ornamental species (Table 1, Figure 1).336 ornamental samples from Mazandaran and 128 samples from Guilan (Northern Iran) provinces were tested using DAS ELISA (Clark and Adames, 1977) for the presence of 6 viruses (Figure 2).Due to lack of antiserum the infectivity tests against *tomato streak virus*-TSV and *tomato mosaic virus*-ToMV were not performed. TSWV infection was recorded to be highest in both the region in Mazandaran and Guilan provinces and ArMV infection level recorded the lowest comparatively. *Ardisia crenata*, *Azali* spp., *Aglonema schott, Bambusa* spp., *Begunia semperflorus, Pandanus veitchii,* Polysia spp., *Bignonia capreolata, Lilium* spp., *Aspidistra elatior, Pinus* spp., *Camellia sinensis, Cordyline* spp., *Lilium longiflorum, Phoenix Canariensis* and *Chamaedorea elegans* were recorded as new ornamental hosts in this research from Iran (Table 1).

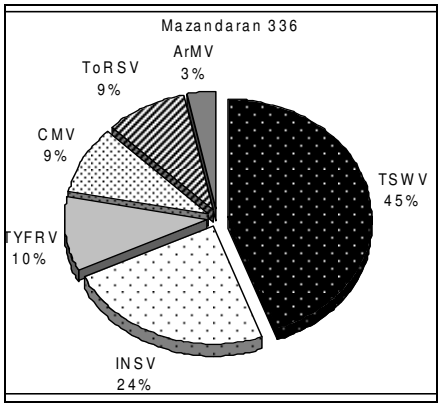
240 plant samples despite of showing some symptoms did not react to any of the 6 viruses tested in Elisa. The highest virus incidence in descending order in

Figure 2. Frequency of the viruses isolated from ornamentals in Guilan and Mazandaran province.





Distribution of viruses in Guilan



Distribution of viruses in Mazandaran

Mazandarn province for TSWV was 83 (17.88%), INSV 47 (10.12%), CMV 32 (6.89%), ToRSV 31 (6.68%), TYRV 23 (4.95%) and ArMV 8 (1.72%) respectively. TSWV and INSV (tospoviruses), CMV(cucumovirus) and ToRSV(nepovirus) were the most prevalent viruses infecting these ornamentals in Mazandaran province ,were as TSWV 43 (38%), INSV 84 (14%), TFRV 37 (9%), CMV and ToRSV 59 (8%) and ArMV 12 (3%) were the most prevalent viruses in Guilan province (Table 1, Figure 2). Of the total of 464 ornamental plant samples tested , 224 samples 48% were virus infected and 240 (51%) were not found to be infected by the above 6 viruses in serological test. The results also indicated of the 46 ornamental species studied, 30 plant species found infected and 16 plant species were shown to be uninfected by the 6 viruses under study (Table 1 ,Figure 2). The results also indicated 65% of ornamental plant species under study were almost infected by at least one of the above 6 viruses. Mechanical inoculation of selected Elisa positive samples for each viruses to some indicator hosts carried by using 0.01M potassium phosphate buffer pH-7 containing 0.15% 2-Mercaptoethanol. TSWV produced necrotic local lesions on *V unguiculata, C. amaranticolor* and *P. hybrida*. Local ring spot lesions followed by systemic mosaic, necrotic ring lesions and leaf abnormality on D metel, N. tabaccum cv.Samsun. TYRV and TSWV (Ghotbi *et al.*,2005) produced similar symptoms upon mechanical inoculation to some indicator test plants, *C. quinoa* and *C. amaranticolor* reacted with local necrotic spots, *N. rustica* local necrotic ring pattern and systemic mosaic, *V. unguicolata* produced local necrotic ring spots, systemic ring pattern on leaves followed by stem and shoot tip necrosis. INSV produced local necrotic lesions followed by systemic wilting on *N. rustica* and *D. metel* inoculated plants. INSV symptom severity on these plants was observed to be higher at warmer condition.

Systemic mosaic was produced by CMV inoculation of cucumber. *C amaranticolor* and *V. unguiculata* reacted with necrotic local lesions and *N. rustica* showing local necrotic lesions plus systemic necrotic ring spots. ToRSV infection on cucumber, *C. amaranticolor*, *C. quinoa* and

V. unguicolata produced necrotic local lesions and bud necrosis in cowpea. *N. rustica* treated with necrotic local lesion and systemic mosaic with ring spots. ArMV inoculated *C. amaranticolor* and *C. quinoa* produced local necrotic ring spots with yellow margin and systemic mottling. *N. tabbacum* cv White Burley and *P. hybrida* produced local necrotic lesions followed by systemic chlorosis. Infectivity of all the inoculated test plants to the viruses was confirmed following by Elisa test.

DISCUSSION

Viruses have always been a major cause of reduced quality and quantity of the worldwide ornamental cut and propagated flowers (Daughtrey *et al*., 1997; EPPO 1999a,b; Mandal *et al*., 2012; Parrella *et al*., 2003). In Iran, high incidence of virus like symptoms is often observed on ornamental plants. In the present study, the incidence and distribution of 6 viruses TSWV, ToRSV, CMV, TYRV, INSV and ArMV infecting propagated ornamentals in greenhouses in Mazandaran and Guilan, Northern provinces of Iran is documented. Six viruses were found infecting propagated ornamentals, with TSWV being the most widespread virus (Table 1). Although CMV incidence of nearly 70% (Ghotbi and Nazerian, 2010) has been reported in some ornamental screen-houses in Tonekabone area of Mazandaran, TSWV (tospovirus) appears to be the most prevalent viruses in ornamentals propagated in Northern Iran. TSWV and TYRV was first reported in 1998 from Varamin area in Tehran (Bananej *et al*., 1998) and TYRV in 2005 (Ghotbi *et al*.,2005) in ornamentals from central region of Iran. TSWV and TYRV are important and damaging viruses infecting ornamentals and different other cultivated crops in Iran (Golnaraghi *et al*., 2001a,b; Golnaraghi *et al*., 2002c,e; Ghorbani *et al*., 2008, 2010; Pourrahim *et al*., 2001; Shahraeen and Ghotbi,2010; Ghotbi 2010b; Ghotbi and Eskandari 2010;Beikzadeh et al.,2012) and elsewhere (Suetic *et al*., 1999). INSV the other tospovirus of lesser spread in Iranian screen-houses was first reported in 2002 (Ghotbi and Shahraeen, 2002) infecting a few ornamental plants (Rose, gazania, Dahlia, Salvia...) in different region of Iran (Shahraeen *et al*., 2002; Ghotbi *et al.,* 2010; Ghotbi and Talebi, 2010). Neverthless the vector identification and transmission of these tospoviruses by different thrips species has also been investigated. TSWV and TYRV high incidence in both the region can be attributed to suitability of environmental condition for the thrips vector activity and presence of different host carrier. Mix cropping is a common practice in Northern region of Iran which may provide suitable condition for the vector colonization and spread to suitable host (Ghotbi *et al.,* 2003; Ghotbi *et al*., 2005; Ghotbi and Baniameri, 2006; Hosseini-nia and Baniameri , 2010). Mix infection of two or more viruses in a particular ornamental species was recorded in our study (Table 1).Natural incidence and infectivity level of three nepoviruses infecting some ornamental was also reported from Markazi and Tehran provinces (Ghotbi and Shahraeen, 2009). ArMV was first reported infecting ornamentals in 2005 (Ghotbi *et al*., 2005). CMV is known to occur in field and screen houses grown plants and has been reported from several regions in Iran (Farzadfar *et al*.,2002; Ghotbi and Shahraeen 2004; Ghotbi, 2010a; Ghotbi and Nazerian, 2010). In this study 464 ornamental samples from 46 different species were tested, a few of which is considered to be used as an open or garden flowers but most were of those to be kept in apartment as an indoor flowers, some lik*e, Ardisia crenata*, *Bambusa* spp., *Begunia semperflorus, Pandanus veitchii, Polysia* spp., *Bignonia capreolata, Cordyline* spp. are reported for the first time as a host infected by these viruses from Iran (Table 1). A percentage of samples 51.72% from symptomatic plants analyzed did not react with the antisera against any of the 6 viruses, the absence of positive reaction may be due to infection of the other viruses, such as AMV, TMV, TuMV, BYMV and of other carlavirus group or latent viruses which are common in ornamental propagated plants. At the final 'production' stage of growing and distributing ornamental plants, losses due to viral infections can range from 10 to 100%, depending upon the virus-host combination (Loebenstein et al., 1995). Viruses of serious consequence recently identified by the floral and nursery industry in key ornamental crops include, but are not limited to: Tospoviruses, Nepoviruses, Potyviruses, Fabaviruses, Closteroviruses, Potexviruses, Carlaviruses, Cucumoviruses, Caulimoviruses (EPPO, 1999a,b; Mandal *et al*., 2012; Parrella *et al*.,2003). There is therefore a need for research on these new and emerging virus and virus-like problems. Different environmentally friendly control measures including screening for resistance to viruses, evaluating local and commercial varieties for virus resistance, application of different therapies, using virus free and certificated vegetative parts and bulbs for cultivation and propagation are reported. Application of different control management to eradicate weeds and minimize insect vector population is also an important factor for control of the virus diseases infecting ornamentals.

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