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INCIDENCE OF VIRUSES AND VECTOR NEMATODES IN THRACE VINEYARDS, TURKEY

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ABSTRACT

A survey on the incidence of nepoviruses and vector nematodes were carried out in main grape growing areas of Thrace Region, European part of Turkey. *Grapevine Leafroll-Associated Virus* and *Grapevine Fleck Virus* were olso investigated in case of combination with any of nepoviruses on grapevine. On this purpose randomly selected commercial vineyards were sampled and total of 434 leaf samples were collected from grapevines exibiting virus like symptoms to confirm the infection by Double antibody sandwich-enzyme linked immunosorbent (DAS-ELISA) assay. Soil samples were also collected from rhizosphere of symptomatic grapevines to evaluate the presence of vector nematode individuals. Six viruses including *Grapevine Leafroll-associated Virus 1* (GLRaV-1), *Grapevine Leafroll-associated Virus 3* (GLRaV-3), *Grapevine Fanleaf Virus* (GFLV), *Grapevine Fleck Virus* (GFkV), *Tobacco Black Ringspot Virus* (TBRV), *Arabis Mosaic Virus* (ArMV) were detected in vineyards. GFLV was most predominant virus and out of 434 tested samples, 277 samples representing 57 % gave positive reactions. Four virus vector nematodes *Xiphinema index, Xiphinema diversicaudatum Xiphinema italiae* and *Longidorus elongatus* were identified in soils.

Keywords*:* Viral diseases, *Xiphinem*a spp., *Longidorus* spp., *Vitis vinifera* L.

INTRODUCTION

Viticulture has a great importance in Turkey's agricultural structure and has a 30% share in total fruit production (Gümüş and Gümüş, 2008). Having favorable ecological conditions and good soil, more than 1200 grape varieties can be grown in almost every part of the country (Gargın et al., 2010; Uzun and Bayir, 2008). More than 1250 varieties are maintained in gene banks at the Tekirdağ National Gerinplasm Repository Vineyard established in Viticulture Research Institute. Approximately 40% of Turkey's grape production is set aside for raisin, 35% for consumption as fresh and 3% for wine production (Gümüş and Gümüş, 2008).

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Tekirdağ, Edirne and Kırklareli are one of the main grape growing provinces of Thrace Region located in the northeastern part of Turkey with total vineyard areas covering a surface of 37.4, 19.5 and 7 ha respectively (Gülcü and Taşeri, 2012; TUIK, 2015). The main cultivars grown in the region are Cabernet Sauvignon, Sauvignon Blanc, Italiae, Alphonse Lavalle, Muscat of Hamburg foreign varieties and Papazkarası, Trakya İlkeren, Erenköy Beyazı, Öküzgözü, Yapıncak local varieties.

Grapevine viruses are most important factors that causes yield losses in vineyards. More than 70 viruses had been reported associated with grapevines through over the world belonging to genuses of Nepovirus, Sadwavirus, Closterovirus, Ampelovirus, Vitivirus, Maculavirus and Foveavirus (Martelli 2006). Yellow foliage, red foliage, shortened internodes, mottling, leaf deformation, stunting are main symptoms of viruses on grapevines.Infected plants also exhibit changes in carbohydrate metabolism and hormonal balances, decrease in photosynthetic potential, increase in respiration rate, reduction of the chlorophyll levels and soluble sugar accumulation in the leaves (Basso, et al, 2017).

These viruses are transmitted from host to host through many vectors including nematodes. Among nematodes only the orders Dorylaimida and Triplonchida have species that are able to transmit viruses to host plants. Two genera *Xiphinema* and *Longidorus* in the family Longidoridae can transmit viruses to healthy grapevines. Virus can be retained by single nematode in 5 minutes and transmitted to healthy plant (Brown et al.,1995; Brown and Robertson, 1990; Hewitt et al., 1958). GFLV is semi-persistently vectored by both juvenile stages and adults of the ectoparasitic nematodes *Xiphinema index* and *Xiphinema italiae* (Demangeat al., 2010). *Arabis mosaic virus* is transmitted by *Xiphinema diversicaudatum*. *Longidorus elongatus* is the other vector vectoring *Raspberry ringspot virus* (Comoviridae: Nepovirus) and *Tomato black ring virus* (Comoviridae: Nepovirus). The only way to prevent virus spread is the use of certified virus free materials and the control of vectors.

The present paper reports the results of an investigation undertaken from 2012 to 2016 to determine the occurrence and distribution of nepoviruses and vector nematodes in main wine growing areas of Thrace region.

MATERIAL AND METHOD

1. Survey of Vineyards

A survey on the incidence of viruses and vector nematodes was carried out between April-October 2014-2016 in main grape growing areas of Tekirdağ, Edirne and Kırklareli Provinces. Virus sampling and investigations were conducted on randomly selected vineyards located in 9 districts and 51 locations. Grapevines sampled were resembling virus like symptoms including

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yellow foliage, red foliage, shortened internodes, mottling and rolling of the leaves. Total of 434 samples were collected from randomly selected vineyards established with 42 different grape cultivars (Common cultivars: Cabernet Sauvignon, Muscat of Hamburg, Merlot, Italiae, Sauvignon Blanc, Alphonse Lavallee, Pinot noir, Chardonnay, Cinsault, Cardinal, Papazkarası, Atasarısı, Erenköy Beyazı, Yapıncak, Kalecik Karası) grafted on several rootstocks including Kober 5BB, 1103 Paulsen, 110 Richter, 41 B and 140 Ruggeri. Nearly 3 leaves from different canes on both arms of each vine were taken, put into bags and labeled. Samples were kept in -20°C till analysis. Soil samples of nearly 1 kg were taken from rhizosphere (0-60 cm soil depth) of each symptoatic grapevine to determine the incidence of vector nematodes.

2. Virus Detection and Identification

Incidence of *Grapevine Fanleaf Virus* (GFLV), *Grapevine Fleck Virus* (GfKV), *Arabis Mosaic Virus* (ArMV), *Tobacco Black Rinspot Virus* (TBRV), *Strawberry Latent RingsotVirus* (SLRSV), Tomato *Ringspot Virus* (TRSV), *Tobacco Ringspot virus* (ToRSV) and *Grapevine Leafroll Associated Virus* (GLRaV) were investigated by Double-antybody sandwich enzyme-linked immuno-sorbent assays (ELISA) (Clark and Adams, 1977) using polyclonal antisera of BIOREBA. All procedures were performed according to the recommendations of the antisera producers. The samples were considered as positive when the absorbance values at 405 nm were greater than three times the average of negative control.

3. Extraction and Identification of Plant Parasitic Nematodes

Nematodes were extracted from 200 g soil samples using combination of decanting and sieving (Brown & Boag, 1988) and centrifugal flotation (Jenkins, 1964). For the species identification nematode individuals from each sample were heat killed at 60ºC for one minute, fixed in TAF solution, transferred to glycerine and mounted on slides by wax-ring method (Seinhorst, 1959). The nematode species were counted and identified with a microscope. Morphometric of species were compared with those of Thorne and Allen, (1950), Loof and Luc (1990), Esser (1974), Loof and Chen. (1999).

RESULTS

Virus Identification in Vineyards

Among 6 viruses tested, 3 viruses were detected (Table 1) in leaf samples and the rate of positive samples was 57 % for *Grapevine Fanleaf Virus* (GFLV), % 0.5 for *Tobacco Black Ringspot Virus* (TBRV) and 0.5% for *Arabis Mosaic Virus* (ArMV) (Table 1).

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The incidence of other viruses was 22 % for *Grapevine Fleck Virus* (GFkV), 4 % for *Grapevine Leafroll-associated Virus 1* (GLRaV-1), 6 % for *Grapevine Leafroll-associated Virus 3* (GLRaV-3). These viruses were found to occur as single or mixed infections of different combinations in individual grapevines (Table 2/ Fig 1). *Strawberry Latent Ringspot Virus* (SLRSV), *Tomato Ringspot Virus* (TRSV), *Tobacco Ringspot virus* (ToRSV) were not detected in any of samples.

Table 1: Incidence of 6 nepoviruses in vineyards of Tekirdağ, Edirne and Kırklareli Provinces

Virus	Infected samples Tekirdağ	Infected samples Edirne	Infected samples Kırklareli	Total infected
Total samples	**358**	**51**	**25**	**434**
GFLV	250	16	11	277
ArMV	2	0	0	2
TBRV	2	1	0	2
ToRSV	0	0	0	0
SLRSV	0	0	0	0
TRSV	0	0	0	0
Total infected	254	17	11	

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Table 2: Prevelance of Grapevine Fleck Virus and Grapevine Leafroll Associated Viruses in vineyards of Tekirdağ, Edirne and Kırklareli Provinces

Virus	Infected samples	Total samples
GfKV	65	434
GLRaV-1	15	
GLRaV-3	23	
GFLV+GFkV	30	
GFkV+GLRaV-1	2	
GFLV+GLRaV-3	3	
GFLV+GLRaV1+GLRaV-3	2	

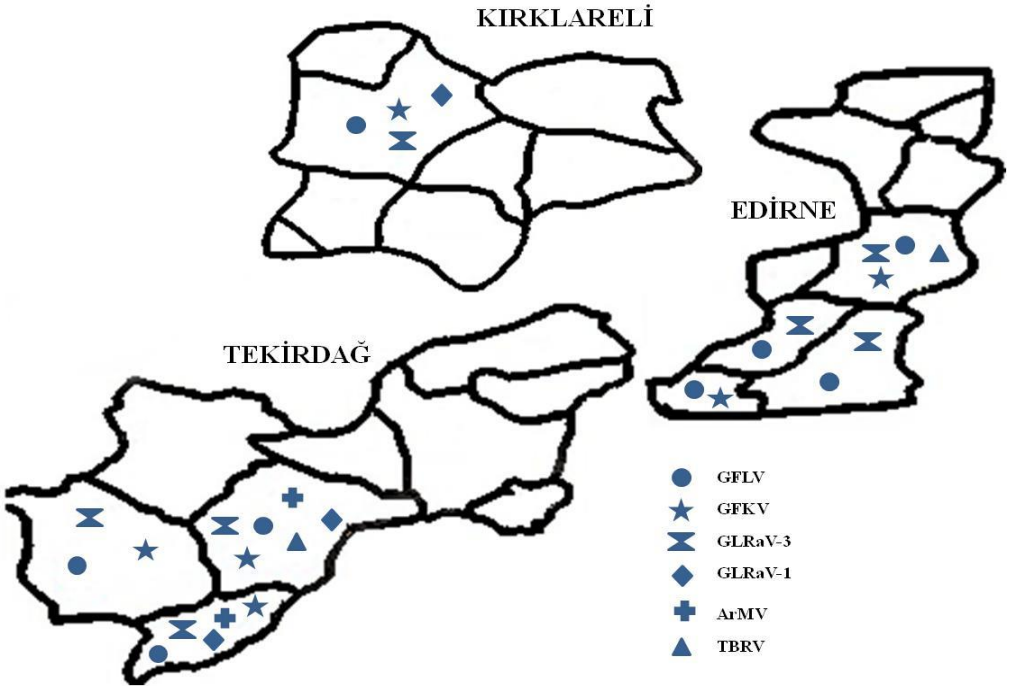

Fig. 1: Distribution of grapevine viruses in three provinces

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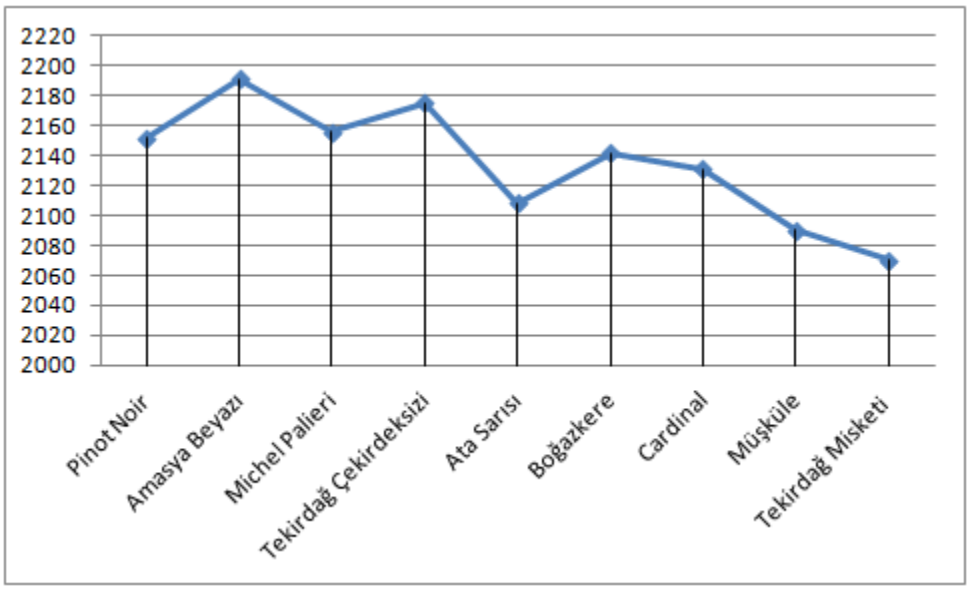
DAS ELISA results confirmed the presence of GFLV in 37 different grapevine varieties, which represent 88 % of total number of varieties. The highest OD (optical density) values at 405 nm was measured in Michel Palieri, Pinot noir, Cardinal foreign varieties and Boğazkere, Amasya siyahı, Atasarısı, Müşküle, Tekirdağ Çekirdeksizi, Tekirdağ Misketi local cultivars. This predominant virus was detected in 69 %, 27%, 44% of samples of Tekirdağ, Edirne and Kırklareli respectively.

Table 3: Average values of some varieties at 405 nm with DAS ELISA.



During survey, malformation, vein banding and yellow mosaic virus strains was detected in grapevines.

The symptoms of malformation virus strain was observed as double nodes, short internodes on the infected grapevines, abnormal brunching asymmetrical leaf blade formation and deformation of leaf as fan shape (Fig 2). On the other hand yellow discoloration was common symptom in yellow mosaic virus strain. Grapevine Fanleaf Virus symptoms were significant in international cultivars such as Michel Palieri, Italia and local cultivars including Trakya İlkeren, Erenköy beyazı, Hatun parmağı and Müşküle.

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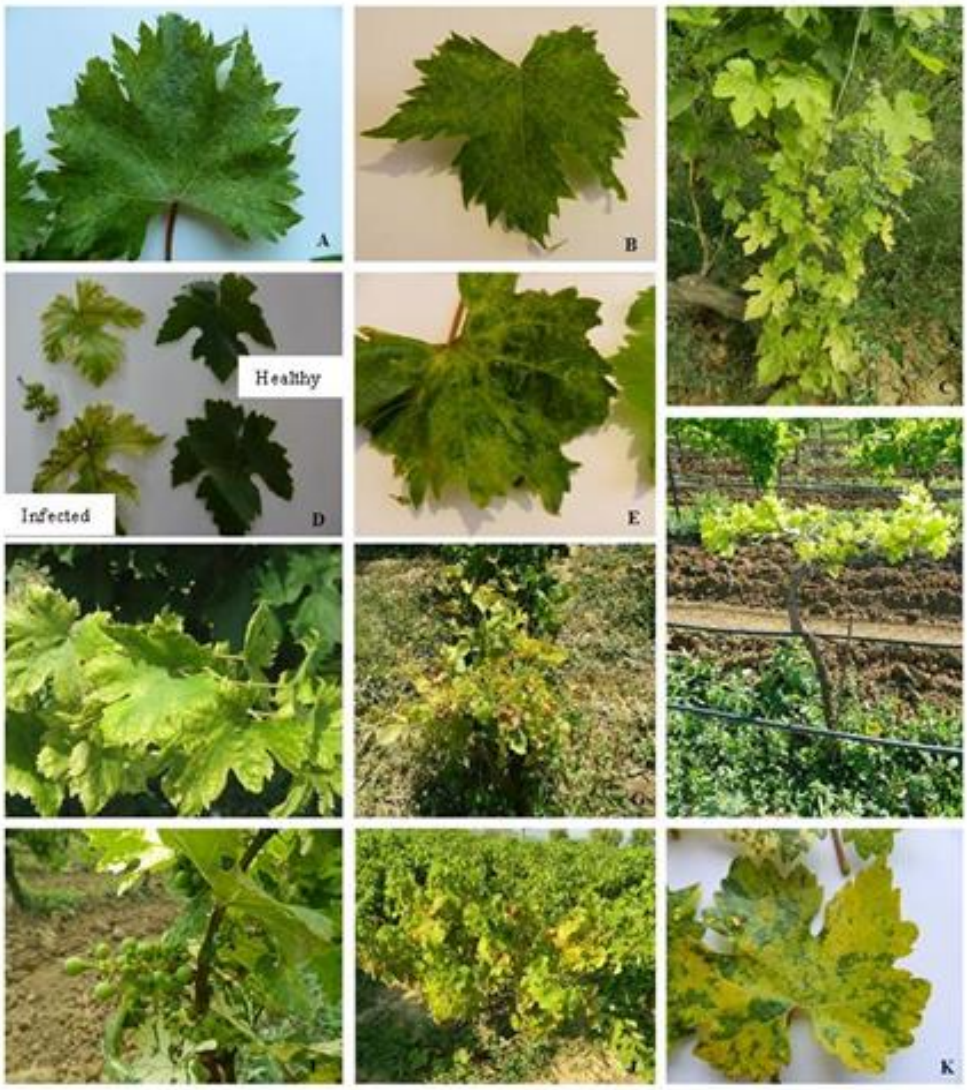

Fig. 2: Symptoms in GFLV infected grapevines

A-B. Malformation strain.

C-K. Yellow mosaic strain

Virus Vector Nematodes in Thrace Vineyards

After species identification of plant parasitic nematodes four virus vector nematodes; *Xiphinema index, Xiphinema italiae, Xiphinema diversicaudatum* and *Longidorus elongatus* were identified in vineyards.

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Xiphinema index (Fig 3) were found in 12% of soils collected from vineyards. The higher population was counted in 40-60 soil depth and was also isolated from soil samples collected from 90-120 soil depth. *Xiphinema italiae* was collected from districts of Tekirdağ and Kırklareli mostly from sandy soils. Distribution rate of *Xiphinema italiae* was 4,6 % for Tekirdağ and 4% for Kırklareli. Density of nematode was 0-10 specimens 200/gr at 0-60 soil depth. *Xiphinema diversicaudatum* was found in only 1 vineyard in Tekirdağ and *Longidorus elongatus* was present in 4 vineyards in Tekirdağ and 1 vineyard in Kırklareli. The higher population of *Longidorus elongatus* was counted as more than 70 individuals at 70-90 cm soil depth.

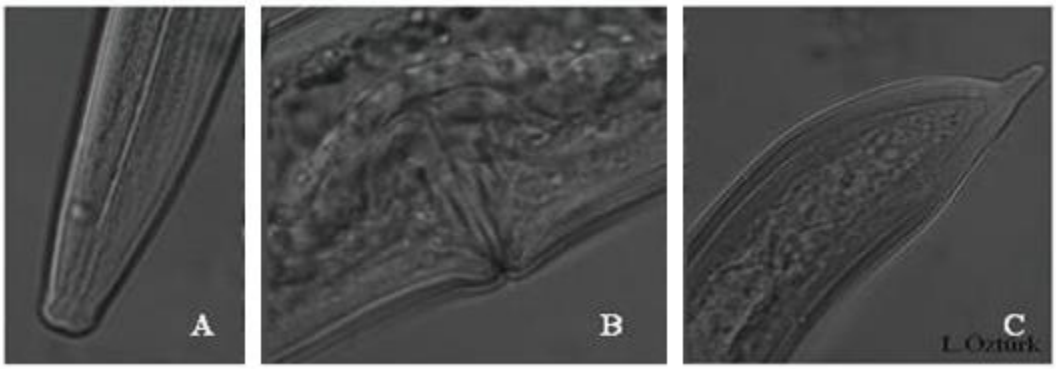

Fig. 4: Xiphinema. index female

A. Anterior region B. Vulva C. Tail

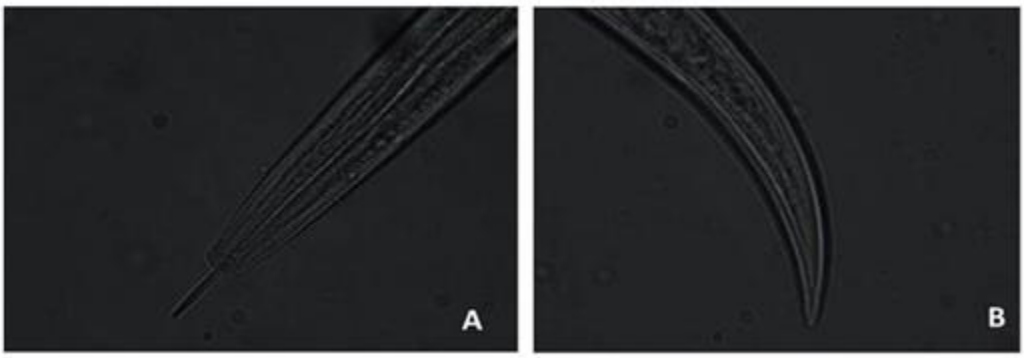

Fig. 5: Xiphinema italiae female

A. Anterior region B. Tail

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According to serogical tests *Grapevine Fanleaf Virus* was detected in 19% of grapevines grown in *Xiphinema index* infected vineyards, Other vector *Xiphinema italiae* was only present in 2% of GFLV infected vineyards and just observed in Tekirdağ. *Longidorus elongatus* was found around the roots of grapevines infected with combination of *Grapevine Fan Leaf Virus* (GFLV) and *Tobacco Black Ringspot Virus* (TBRV). *Xiphinema diversicaudatum* was just isolated from one vineyard established with local variety having ArMV infection. (Fig 4).

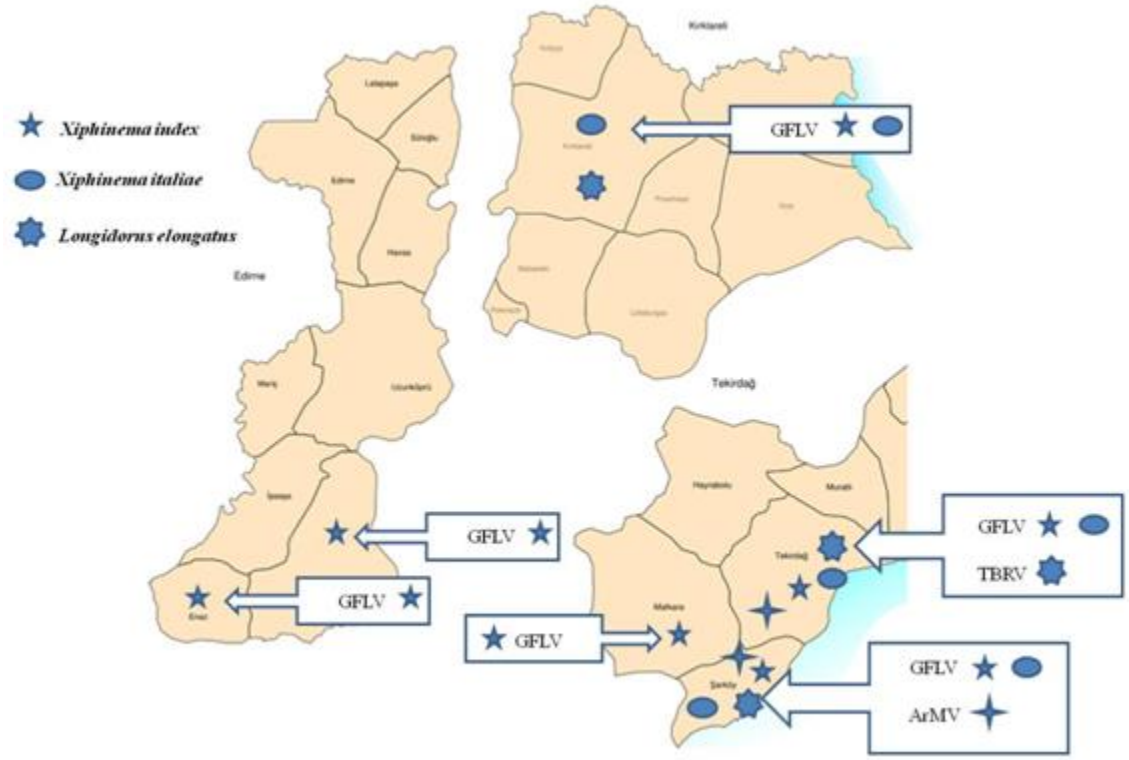

Fig. 4: Distribution of *Xiphinema index, Xiphinema italiae, Xiphinema diversicaudatum, Longidorus elongatus* and related viruses in Thrace vineyards

DISCUSSIONS

Grapevine is susceptible to many pests and diseases. Viruses were considered as one of the most important agents due to lack of chemical control and difficulties in vector management. Within more than 70 viruses reported in grapevines, only the grapevine leafroll, rugose wood complex, fanleaf and fleck diseases cause serious infections affecting plant growth resulting in serious yield and quality loss. *Grapevine fanleaf virus* (GFLV) is one of the most severe virus diseases of grapevines worldwide. This nepovirus is transmitted by two longidorid nematodes *Xiphinema index* and *X. italiae* (Halbrend.,1993). GFLV was the most widely distributed virus currently

detected in our study followed by GfKV, GLRaV-3 GLRaV-1, TBRV, ArMV. These viruses were previously found in different parts of Turkey (Gürsoy, 1988; Akbas ve Erdiller, 1993; Yılmaz,1997; Cıgsar ve ark., 2002; Akbas ve ark, 2007; Koklu et al 1998).

Vineyards established with varieties of Pinot noir and *Alphonse Lavallee* were mainly infected with GFLV and GLRaV viruses. Pinot noir grapevine is reported susceptible to virus infections (Haeger, 2004) and in our study all samples from this variety was infected with at least one virus especially GLRaV-3.

Becides, vector nematode survey revealed the presence of *Xiphinema index, Xiphinema italiae, Xiphinema diversicaudatum* and *Longidorus elongatus* in vineyards. *Xiphinema index* was extracted from rhizosphere of 45 virus infected grapevines in Tekirdağ, 2 samples at Edirne 1 sample collected from Kırklareli Provinces. The prevalence of X. index makes this species a severe threat for virus transmission to healthy plants if Grapevine Fanleaf Virus (GFLV) is detected in the vineyard.

But according to all results of our study the relatively high incidence of *Grapevine Fanleaf Virus* and low incidence of nematodes suggests that nematode is not the only reason of virus spread in the region. The same observations were made for *Grapevine Leafroll Aassociated Viruses* of which the vector mealybugs did not observed in every infected grapevine. The reason for higher prevalence of viruses in the Region is the grafting with infected cuttings. Thus the use of virus free plant material for grafting and certified rootstocks for planting is essential for the prevention of new infections.

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