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TRANSMISSION OF ALFALFA MOSAIC VIRUS THROUGH *SUBCOCCINELLA VIGINTIQUATUORPUNCTATA* (L.) (COLEOPTERA: COCCINELLIDAE) IN ALFALFA GROWING AREAS IN TÜRKİYE

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ABSTRACT

Alfalfa *Medicago sativa* L. (Fabaceae: Leguminosae) is an important fodder crop due to its highly nutritious animal feed and potential to adapt various environmental conditions. The average alfalfa yield in Türkiye is considerably higher than the world's average. Several diseases and pests exert negative impacts on alfalfa yield. This study investigated the status of the alfalfa mosaic virus (AMV) and the presence of insect species that could be new vectors for AMV in the alfalfa growing areas of Zonguldak and Bartın provinces in Türkiye. AMV is non-persistently transmitted by aphid species, but it is also known to be transmitted by pollen and seeds. The most prevalent harmful insect species in the studied area were *Subcoccinella vigintiquatuorpunctata* (L.) (Coleoptera: Coccinellidae), and *Gonioctena fornicata* (Brüggeman) (Coleoptera: Chrysomelidae). The AMV was prevalent in the tested plants at a rate of 85.00%, with infection rates of 80.55% in mature larvae of *S. vigintiquatuorpunctate*, 80.00% in pupae, and 96.77% in adults. When looking at the relationship of AMV with common pest species, AMV was not detected in all life stages of *G. fornicata*. In contrast, AMV was detected in all life stages of *S. vigintiquatuorpunctata* except eggs. This study is the first in the world to detect the presence of AMV in *S. vigintiquatuorpunctata*. However, further studies are needed to determine whether *S. vigintiquatuorpunctata* transmits AMV persistently, semi-persistently, or non-persistently.

Keywords: Forage crops, 24-spot ladybird, Molecular detection, Insect Vector, Prevalence

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INTRODUCTION

Alfalfa (*Medicago sativa*) is a perennial forage crop known as the queen of fodders. It is cultivated on>30 million hectares' intemperate regions of the world (Acharya *et al.,* 2020; Li *et al.,* 2020; Tucak *et al.,* 2020). It is regarded as a superior forage crop and contains high amount of protein, minerals, vitamins, and a balanced amino acid composition (Wang *et al.,* 2015). Alfalfa is a natural host for different plant viruses, like several other crops (Samac *et al.,* 2015).

Thirty-one viruses are known to infect alfalfa crops (Usta and Güller, 2020). Natural plant openings or wounds are required for the pathogens to penetrate the plant. Insects are part of the disease complex because feeding wounds constitute the entry point for plant pathogens (Willsey *et al.,* 2017). Several modes of virus transmission exist, including plant contact, vegetative reproduction, and vector-mediated transmission. Insects acquire and spread viruses by feeding on infected plants, vomiting sap and virus particles, and depositing them in chewing wounds; the efficiency and duration of virus retention varies among insect species. Insects are vital virus vectors and more than 70 species are known to transmit economically important crop viruses. They come from families such as Chrysomelidae, Coccinellidae, Curculionidae, and Meloidae. Both adult insects and larvae can effectively transmit plant viruses thanks to their feeding behavior and modes of transmission that facilitate the spread of the virus within plants (Wielkopolan *et al.,* 2021).

Alfalfa mosaic virus (AMV) reduces the alfalfa crop's production, quality durability, and causes significant economic damage. The AMV has the largest range of hosts among plant viruses as it infects 698 species belonging to 167 genera in 71 families (Hull, 1969; Edwardson and Christie, 1997). However, the main hosts for the AMV are *M. sativa*, *Phaseolus vulgaris* L., *Pisum sativum* L., and *Nicotiana tabacum* L. (Mangeli *et al.,* 2019). The AMV infection in alfalfa causes serious crop losses with mosaic spots, yellowing in the form of oak leaves, vein banding, and different deformities in addition to severe stunting. Several studies have indicated that AMV causes 30 to 100% yield loss in alfalfa, depending on ecological conditions and the strain of the virus (Zschau and Janke, 1962; Jaspars and Bos, 1980;

Van Regenmortel and Pinck, 1981). The AMV is a multipartite virus composed of three linear RNAs (RNA1, RNA2, and RNA3) and a non-infective subgenomic RNA (sgRNA4). The four viral RNAs are encased in individual bacilliform particles. The RNA1 and RNA2 are viral replicas subunits. These contain a single open reading frame (ORF) encoding (P1 and P2, respectively). The RNA3 contains two ORFs encoding movement protein and coat protein (CP). (Scott *et al.,* 1998; Moradi and Mehrvar, 2021).

The virus can spread rapidly from one plant to another. It is known that the AMV is non-persistently transmitted by at least fourteen aphid species. The prevalent aphid species in Zonguldak and Bartın province of Türkiye are *Acyrthosiphon pisum* (Harris), *Aphis gossypii* Glover, and *Therioaphis* (*Pterocallidium*) *trifolii* (Monell) (Hemiptera: Aphididae) (Barış *et al.,* 2017). Apart from the aphid species, the AMV has also been reported to transmitted by pollen, *Cuscuta* spp. and seeds (Frosheiser, 1974; Hemmati and McLean, 1977).

Larvae and adults of 24-spot ladybird, *Subcoccinella vigintiquatuorpunctata* (L.) (Coleoptera: Coccinellidae), feed on the leaves of host plants. They eat the lower epidermis and parenchyma of the leaves, whereas the upper epidermis remains in the form of a membrane (Barış, 2021; Barış, 2022). The damaged leaves appear in the form of lace, which can cover the whole plant under severe infestation. Alfalfa forage yield can be reduced by 40 to 60% as a result of the 24-spot ladybird infestation (Keresi and Sekulic, 2005). The ladybird adults overwinter in the soil near host plants (Marriner, 1927; Tanasijevic, 1958; Richards *et al.,* 1976; Wheeler and Henry, 1981; Baldwin, 1988). The other significant pest alfalfa leaf beetle *Gonioctena fornicata* (Brüggemann) (Coleoptera: Chrysomelidae) is substantial pests of alfalfa crop. It is a widespread species in Asia, Siberia, Europe, North Africa, Russia, and Western Asia (Horion, 1961).It has more than 70 hosts, especially in alfalfa (Richards *et al.,* 1976). Bodenheimer (1958) reported that its presence in Türkiye for the first time and named it as 'Clover beetle'. It causes significant crop losses, especially in legumes. The larvae and adults of *G. fornicata* cause significant yield losses in alfalfa. Barış (2021) reported that larvae and adults feed on young shoots, leaf buds, flowers, leaves and the tips of alfalfa stems. The alfalfa leaf beetle was firstly reported by Alkan (1946) in Türkiye. Afterwards, Bodenheimer (1958) regarded it alfalfa leaf beetle and stated that there is an epidemic risk of the pest which would exert significant economic damages.

This study, it was aimed to determine the infection rate of AMV in alfalfa plants, which is a problem in alfalfa cultivation areas in Türkiye and causes yield losses, as well as the detection of pest species in alfalfa cultivation areas and to test these detected pest species for the possible presence of AMV virus.

MATERIALS AND METHODS

Survey studies: Field sampling was carried out in alfalfa fields in the Western Black Sea Region of Türkiye (Bartın and Zonguldak provinces) every two weeks in April, May, and June in 2013-2014 (Table 1). Eggs were collected in April, mature larvae in May, pupae in May-June, and adults in June. The altitude of the surveyed alfalfa fields varied between 55 and 575 m. The green parts of the alfalfa plant were carefully examined with macro-observations, and the presence of any life stage of the harmful species (Çalışkaner *et al.,* 1989; Tamer *et al.,* 1997) and the symptoms caused by AMV in the plant were recorded. As a result of macro-observation, plant parts and insects including, eggs, larvae, pupae and adults of the pests were taken in a paper bag and brought to the plant pest laboratory of the Plant Protection Central Research Institute. The insects were separated according to their stages and taken into tubes containing 99% ethanol in the laboratory. These tubes were then labeled and stored in the +4°C refrigerator, and the plant parts were stored in the deep freezer at -20°C for further analysis.

Eggs, mature larvae, and adult stages of the 24-spot ladybeetle observed on alfalfa plants during the current study are shown in Fig. 1.

Different life stages of (egg, mature larvae, and adult) the harmful insect species were recorded and collected with the infected plant parts. The collected samples were transferred to eppendorf tubes containing 99% ethanol. Afterward, these tubes were brought to the laboratory by placing them in polyethylene bags tagged with the collection date, location, and the number of host plants. The pupae of the pest were cultured in breeding cages at 25±2°C temperature, 16:8 light: dark period, and 65±5% relative humidity under laboratory conditions. The pupae were observed for 8 every hour intervals and taken into 99% ethyl alcohol for analysis before they started feeding.

The prevalence of pests was recorded, and the infection rate calculated by Bora and Karaca, (1970).

$$Prevalence\ rate = \frac{\text{Sampled area}}{\text{Total area}} x100$$

Total RNA extraction: The alfalfa plant samples and different life stages (eggs, mature larvae, pupae, and adults) of the harmful insect species collected during surveys were analyzed simultaneously. In insects, each individual was considered separately, and in eggs, each cluster was considered a single sample. RNA isolations for all samples were made according to the silica gel method (Foissac *et al.,* 2001). The concentrations of the obtained total RNAs were adjusted using Nanodrop (Thermo Scientific, USA).

Table 1. Information on the geographical coordinates of the province and districts where the materials were obtained.

Bartın province	Zonguldak province	
Merkez	**Yılanlıca**	**Eceler**
41°32'41.0"N 32°20'38.0"E 95 m	41°16'07.7"N 31°58'32.7"E 142 m	41°20'39.4"N 32°01'30.1"E 115 m
41°30'33.0"N 32°21'02.0"E 88 m	41°16'14.2"N 31°58'17.7"E 133 m	41°20'30.8"N 32°00'48.7"E 153 m
41°33'14.8"N 32°20'09.3"E 55 m	41°16'12.5"N 31°57'57.7"E 153 m	41°20'03.5"N 32°00'32.1"E 191 m
41°28'28.5"N 32°17'53.1"E 76 m	**Yassıören**	**Kayıkçılar**
41°28'51.6"N 32°18'13.7"E 84 m	41°17'44.5"N 31°56'09.7"E 443 m	41°22'44.2"N 32°05'35.5"E 66 m
Ulus	**Bölücek**	41°23'06.3"N 32°05'43.6"E 70 m
41°32'52.8"N 32°36'21.3"E 126 m	41°18'42.6"N 31°58'54.3"E 416 m	41°23'30.8"N 32°05'38.0"E 57 m
41°31'56.2"N 32°35'37.1"E 175 m	41°19'01.2"N 31°59'06.5"E 390 m	**Nebioğlu**
41°32'21.6"N 32°38'00.8"E 197 m	**Karaman**	41°26'08.9"N 32°15'09.3"E 102 m
41°37'58.9"N 32°38'44.6"E 457 m	41°21'34.0"N 31°58'57.7"E 240 m	41°26'33.8"N 32°15'21.6"E 110 m
41°38'56.4"N 32°41'03.0"E 575 m	41°21'25.9"N 32°00'43.1"E 141 m	41°26'37.1"N 32°14'39.5"E 93 m
Amasra	41°21'09.8"N 32°00'56.6"E 131 m	
41°43'22.8"N 32°26'47.3"E 182 m		
41°43'20.8"N 32°26'56.4"E 187 m		

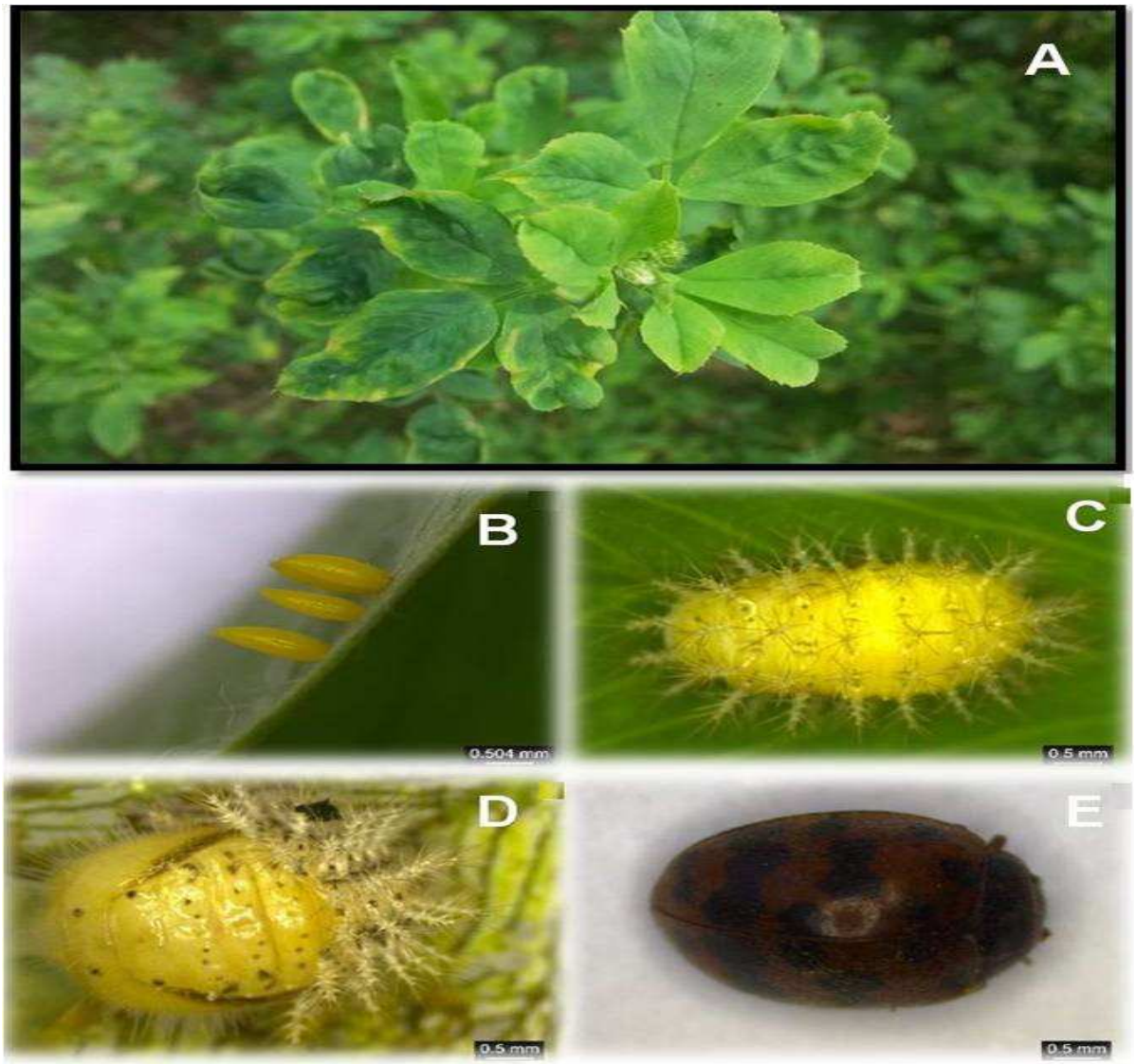

Figure 1. Alfalfa plant with AMV infection symptoms (a), eggs (b), larva (c), pupa (d) and adult (e) of *Subcoccinella vigintiquatuorpunctata*

RT-PCR (Reverse Transcription Polymerase Chain Reaction): The resulting total RNAs were analyzed in a single-stepped RT-PCR (Xu and Nie, 2006). The primer pairs (AMV-F 5′ -CCATCATGAGTTCTTCACAAAAG-3′ and AMV-R 5′ -TCGTCACGTCATCAGTGAGAC-3′) were used for the amplification of the coat protein genome region of AMV. The RT-PCR was performed according to following conditions: total volume of 25 µl PCR mix: 8 µl 5X GoTaq Flexi buffer, 1.2 µl $MgCl_2$ (25 mM), 0.7 µl dNTP (10 mM), 0.25 µl GoTaq polymerase enzyme (5 U µl), 1 µl forward primer (10 µM), 1 µl Reverse primer (10 µM), 0.2 µl Reverse-transcriptase enzyme (200 U/µl), 0.2 µl RNase inhibitor (5000 U/ml), 2 µl RNA prepared to total volume with sterile water without nuclease. The reaction conditions for the one-step RT-PCR were; 60 min at 37 °C, 5 min at 94 °C, 30 s at 94 °C, 30 s at 57 °C, 45 s at 72 °C, and 10 minutes at 72 °C. The amplicons obtained with the primer pair were run on a 1.5% agarose gel prepared with Pronosafe (Conda, Madrid, Spain) DNA dye at 80 V for 60 minutes and visualized under UV transilluminator.

RESULTS AND DISCUSSION

During the research, two different harmful species, *S. vigintiquatuorpunctata* and *G. fornicata*, were detected in alfalfa fields. The prevalence rate of *G. fornicate*, and *S. vigintiquatuorpunctata* was 100%. Similarly, the AMV prevalence rate in the alfalfa samples was 85.00% (Table 2).

Due to the high level of AMV infection in the same fields, the relationship between the pest and the virus was evaluated. As a result of RT-PCR studies, while AMV was not detected in all life stages of *G. fornicata*, the presence of AMV was detected in all life stages of *S. vigintiquatuorpunctata* except egg (Fig. 2).

Table 2. The RT-PCR testing of harmful insect species recorded from alfalfa fields in Zonguldak and Bartın provinces during 2013-2014

Locations		*Suboccinella vigintıquatuorpunctata*				*Gonioctena fornicata*			Alfalfa
		Number of							
		Eggs	Larvae	Pupae	Adults	Eggs	Larvae	Adults	Plant samples
Zonguldak	Yassıören	75	15	18	25	60	18	14	15
	Bölücek	69	18	22	34	82	12	18	12
	Karaman	82	14	16	32	58	10	22	17
	Eceler	60	12	14	38	54	22	20	13
	Kayıkçılar	36	20	12	33	72	20	19	18
	Nebioğlu	62	16	18	42	64	17	15	14
Bartın	Merkez	68	17	20	40	66	14	14	8
	Ulus	74	14	16	37	48	11	18	13
	Amasra	34	18	19	29	42	10	12	10
	Total*	560	144	155	310	546	134	152	120
AMV-positive samples		0	116	124	300	0	0	0	102
AMV infection rate (%)		0.00	80.55	80.00	96.77	0.00	0.00	0.00	85.00

*Total number of eggs, insects and alfalfa plants collected during the surveys

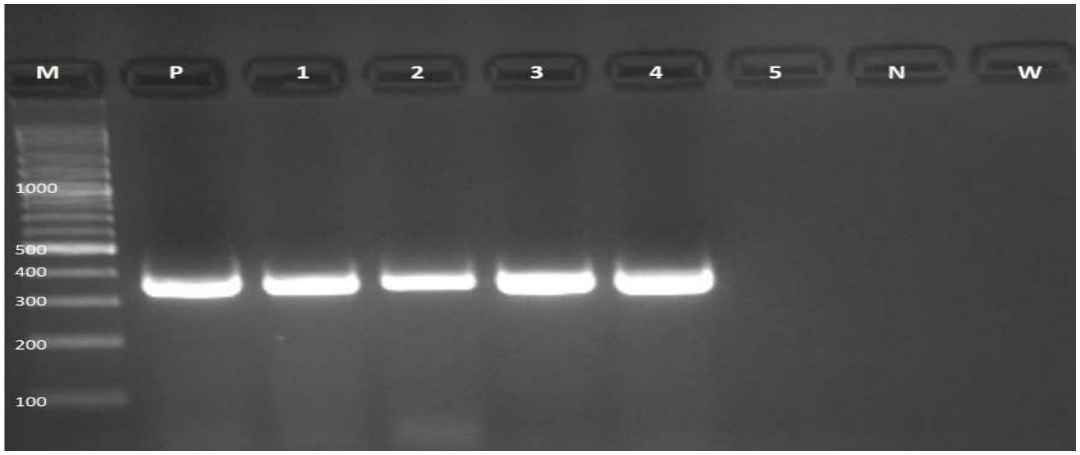

Figure 2.Agarose gel electrophoresis of RT-PCR amplification product for *Subcoccinella vigintiquatuorpunctata* using AMV-F/R primer pairs. In the upper figure panel; M=DNA 100 bpladder, P= positive control, 1=mature larvae, 2= alfalfa plant sample, 3=pupae, 4=adult, 5=egg, N= negative control and W= water control.

The RT-PCR confirmed the presence of AMV in 80.55% of the tested *S. vigintiquatuorpunctata* mature larvae, 80.00% of pupae and 96.77% of the adults. The highest AMV presence in adults can be linked to adult flights and feeding from different plants. Previous research has demonstrated that an insect can obtain a virus from plant tissue through a single bite. However, the effectiveness of virus acquisition rises as the insect engages in more extensive feeding, as noted by Fulton and Scott in 1977. Moreover, insects are capable of ingesting and transmitting the virus within a matter of seconds, and the duration of virus retention varies between 1 and 10 days, contingent upon the insect species, according to Agrios (2008).

On the other hand, no natural enemies of *S. vigintiquatuorpunctata* were found in alfalfa fields in the present study. However, the larval parasitoid *Tetrastichus* sp. (Hymenoptera: Eulophidae) has been reported from other parts of the world (Wheeler and Henry, 1981). The presence of AMV in all locations where *S. vigintiquatuorpunctata* was discovered is believed to be attributed, in part, to the absence of natural predators for this insect within the examined fields. Analysis of *S. vigintiquatuorpunctata* larvae for the presence of AMV virus showed that the virus was also present in larvae. In parallel with this study, similar studies on the presence of viruses in larvae have been recorded in the literature. Nault (1978) found that larvae of *Oulemamelanopus* (Chrysomelidae) transmit maize chlorotic mottle virus (MCMV, Tombusviridae) more efficiently than adults. Additionally, *O. melanopus* and *O. gallaeciana* can effectively transmit cocksfoot mottle virus (CfMV, *Solemoviridae*) up to 15 days after acquisition (Catherall, 1987). Furthermore, Musser *et al.,* (2003) noted that *Epilachna varivestis* (Coccinellidae) exhibits a preference for feeding on plants that have undergone visual changes due to infections by bean pod mottle virus (BPMV, Secoviridae) and southern bean mosaic virus (SBMV, Solemoviridae). The observations of the current study suggest that the pest ingests the virus through diet during feeding. The non-infected eggs indicate that AMV is not transmitted from one generation to the other in *S. vigintiquatuorpunctata*.

In the observations made for *G. fornicata*, it was determined that only adult individuals were fed with alfalfa, which caused yield losses (Barış *et al.,* 2021). However, the AMV was detected in *G. fornicata* feeding plants, but the virus was not found in any stage of this pest. The ability of an insect to serve as a vector has been reported to depend on several factors, including specific molecular interactions between the virus and the insect host (Chen *et al.,* 2015). As stated in Chen *et al.,* (2015), the interaction between the non-structural protein of a virus and the cytoplasmic actin of leafhoppers is related to insect vector specificity. In this study, we did not find AMV in *G. fornicata*, which is thought to be due to molecular incompatibility between host and pathogen.

It is known that aphids are the only vectors for transmission of AMV. There are >20 aphid species that can transmit AMV. Among these species, *Aphis pisum, A. craccivora, A. fabae,* and *Myzus persicae* are the most important and transmit AMV non-persistently (Edwardson and Christie, 1997). Although aphid species were not prioritized in this study, observations showed that the density of some aphid species was very low in samples collected from alfalfa fields. It can be said that natural enemies suppress aphid populations. Aphid plays an important role in transmitting of AMV; however, no pest management strategies, the presence of natural enemies, and their activities lower the aphid population (Katis *et al.,* 2007). The prevalence of AMV in alfalfa causes significant damage. Several natural enemies, especially those in the Coccinellidae family, significantly suppress aphids population in Türkiye (Kök *et al.,* 2020). Kaygın and Kaptan (2017) reported that 14 Coccinellidae species from Bartın province and the species with the highest prevalence rates were; *Coccinella septempunctata* (L.), *Harmonia axyridis* (Pallas), *Scymnusquadri guttatus* (Capra), *Halyziasedecim guttata* (L.), *Oenopiacon globata* (L.), *Propyleaquatuordecim punctata* (L.), and *Adaliade punctata* (L). The *C. semptempunctata* was commonly observed from both provinces during the surveys. In alfalfa fields, no pest management strategy was favored that was considered effective in promoting beneficial fauna and suppressing aphid populations.

In conclusion, this is the first report that the AMV was only recorded from *S. vigintiquatuorpunctata* in the current study. Insects can serve as vectors for the transmission of viruses, including plant viruses. The transmission of viruses by insect vectors can occur through circulative or non-circulative modes, depending on the specific interactions between the virus and the insect host. Transmission studies under controlled conditions are needed to confirm the vector status of the insect. Understanding these interactions is crucial for developing strategies to control the spread of plant diseases and protect plants from viral infections.

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REFERENCES

Acharya, J.P., Y. Lopez, B.T. Gouveia, I. De Bem Oliveira, M.F.R. Jr. Resende, P.R Muñoz and E.F. Rios (2020). Breeding alfalfa (*Medicago sativa* L.) adapted to subtropical

agroecosystems. Agronomy. 10(742): DOI: 10.3390/agronomy10050742

Agrios, G.N., (2008). "Transmission of plant diseases by insects," in Encyclopedia of Entomology. ed. J. L. Capinera (Dordrecht: Springer Netherlands), 3853–3885.

Alkan, B., (1946). Agricultural entomology. T.C. Ministery of Agriculture, Ankara Higher Institute of Agriculture textbook 31; Higher Agricultural Institute Printing House, Ankara (Türkiye). 232 p.

Baldwin, A.J., (1988). Biological observations on *Subcoccinella vigintiquattuorpunctata* (L.) (Col., Coccinellidae). Entomologist's Monthly Magazine. 124(1484-1487): 57-61.

Barış, A., I. Özdemir, C. Yücel and N. Gök (2017). Harmful Aphididae species (Hemiptera: Aphididae) in the alfalfa areas of Zonguldak. September: 4-8, 10th ISA Nevşehir, Cappadocia, Türkiye, Poster presentation.

Barış, A., C. Yücel and N. Gök (2021). Distribution, density and population monitoring of *Gonioctena fornicata* (Brüggemann, 1873) (Coleoptera: Chrysomelidae) in lucerne fields of Bolu, Zonguldak and Bartın provinces. Plant Protection Bulletin. 61(1):15-20. DOI: 10.16955/bitkorb.796566

Barış, A., (2021). General information about *Subcoccinella vigintiquatuorpunctata* Linnaeus, 1758 (Coleoptera: Coccinellidae). Recent Biological Studies; Chapter 7: 143-159 p. ISBN: 978-625-8061-21-5.

Barış, A., (2022). Age-Stage, Two-Sex Life Tables of the 24 Spot Ladybird [(*Subcoccinella vigintiquatuorpunctata* (Coleoptera: Coccinellidae)]. Brazilian Archives of Biology and Technology. 65: 1-10.e22210841. DOI: 10.1590/1678-4324-2022210841

Bodenheimer, F.S.A., (1958). Study on insects that are pests to agriculture and trees in Turkey and the management against them. Bayur printing house; Ankara (Türkiye). 175 p. (in Turkish).

Bora, T. and I. Karaca (1970). Measurement of disease and damage in cultivated plants. Ege University, Faculty of Agriculture Auxiliary Textbook, No. 167, İzmir (Türkiye). 8 p. (in Turkish).

Catherall, P.L., (1987). Effects of barley yellow dwarf and ryegrass mosaic viruses alone and in combination on the productivity of perennial and Italian ryegrasses. Plant Pathology. 36:73-78. DOI: 10.1111/j.1365-3059.1987.tb02179.x

Chen, Q., H. Wang, T. Ren, L. Xieand and T. Wei (2015). Interaction between non-structural protein pns10 of rice dwarf virus and cytoplasmic actin of leafhoppers is correlated with insect vector specificity. J. General Virology, 96(4): 933-938. DOI: 10.1099/jgv.0.000022

Çalışkaner, S., N. Dörtbudak and A. Has (1989). Survey studies on the potato tuber moth (*Phthorimaea operculella* Zeller) in central anatolia. Plant Protection Bulletin. 29(1): 65-79. https://dergipark.org.tr/tr/pub/bitkorb/issue/3646/48610.

Edwardson, J.R. and R.G. Christie (1997). Viruses infecting peppers and other solanaceous crops. Volume I. University of Florida. Gainesville, Florida. 336 p.

Foissac, X., L. Svanella-Dumas, M.J. Dulucq, T. Candresse, P. Gentitand and M.F. Clark (2001). Polyvelent detection of fruit tree tricho, capillo and fovea viruses by nested RT-PCR using degenerated and inosine containing primers (PDO RT-PCR). Acta Horticulturae. 550: 37-43. DOI: 10.17660/ActaHortic.2001.550.2

Frosheiser, F.I., (1974). Alfalfa mosaic virus Transmission to Seed through Alfalfa Gametes and Longevity in Alfalfa Seed. Phytopathology. 64: 102–105.

Fulton, J.P. and H.A. Scott (1977). Bean rugose mosaic and related viruses and their transmission by beetles. Fitopatol. Bras. 2: 9–16.

Hemmati, K. and D.L. McLean (1977). Gamete-seed Transmission of Alfalfa mosaic virus and its effect of Seed Germination and Yield in Alfalfa Plants. Phytopathology.67: 576-579.

Horion, A., (1961). A Faunistik der Mitteleuropischen Kafer. Band VIII. Uberlingen- Bodensee, Komissionsverlag Buchdruckerei Ang. Feysel, 283-365.

Hull, R., (1969). Alfalfa mosaic virus. Adv. Virus Res.15:365–433.

Jaspars, E.M.J. and L. Bos (1980). Alfalfa mosaic virus AAB Description of Plant Viruses. CMI/AAB Descriptions of Plant Viruses, No. 229 p.

Kaygın, A.T. and U.S. Kaptan (2017). Coccinellidae (Insecta: Coleoptera) Species of Bartın Province. J. Bartın Faculty of Forestry. 19(2): 227-236. DOI: 10.24011/barofd.356628

Keresi, T. and R. Sekulic (2005). Alfalfa beetle and alfalfa lady bird beetle-important defoliators of perennial fodder legumes. Biljni Lekar (Plant Doctor). 33(5): 509-516.

Katis, N.I., J.A. Tsitsipis, M. Stevens and G. Powell (2007). Transmission of plant viruses. In H.F. van Emden & R. Harrington (Eds.), Aphids as crop pests, CABI, Wallingford (United Kingdom). 353-390 p. DOI: 10.1079/9780851998190.0353

Kök, Ş., Ž. Tomanović, Z. Nedeljković, D. Şenal and İ. Kasap (2020). Biodiversity of the natural enemies of aphids (Hemiptera: Aphididae) in

Northwest Turkey. Phytoparasitica. 48(1): 51-61. DOI: 10.1007/s12600-019-00781-8

Li, D., D. Liu, M. Lv, P. Gao and X. Liu (2020). Isolation of triterpenoid saponins from *Medicago sativa* L. with neuroprotective activities. Bioorganic & Medicinal Chemistry Letters. 30 (4), 126956. DOI: 10.1016/j.bmcl.126956

Mangeli, F., H. Massumi, F. Alipour, M. Maddahian, J. Heydarnejad, A. Hosseinipour, M.H. Amid-Motlagh, M. Azizizadeh and A. Varsani (2019). Molecular and partial biological characterization of the coat protein sequences of Iranian Alfalfa mosaic virus isolates. J. Plant Pathology. 101(3): 735-742. DOI: 10.1007/s42161-019-00275-w

Marriner, T.F., (1927). Observations on the life history of *Subcoccinella24·punctata*. Entomologist's Monthly Magazine.63:118-122.

Moradi, Z. and M. Mehrvar (2021). Whole-genome characterization of alfalfa mosaic virus obtained from metagenomic analysis of Vinca minor and Wisteria sinensis in Iran: With implications for the genetic structure of the virus. The Plant Pathology J. 37(6): 619. DOI: 10.5423/PPJ.OA.10.2021.0151

Musser, R.O., S.M. Hum-Musser, G.W. Felton and R.C. Gergerich (2003). Increased larval growth and preference for virus-infected leaves by the Mexican bean beetle, *Epilachna varivestis* Mulsant, a plant virus vector. J. Insect Behav. 16: 247–256. DOI: 10.1023/A:1023919902976

Nault, L.R., (1978). Transmission of maize chlorotic mottle virus by chrysomelid beetles. Phytopathology 68:1071-1074. DOI: 10.1094/Phyto-68-1071

Richards, A.M., R.D. Pope and V.F. Eastop (1976). Observations on the biology of *Subcoccinellavigintiquattuor-punctata* (L.) in southern England. Ecological Entomology. 1(3): 201-207. DOI: 10.1111/j.1365-2311.1976.tb01223.x

Samac, D.A., L.H. Rhodes and W.O. Lamp (2015). Compendium of Alfalfa Diseases and Pests, 3rd Edn, APS Press American Phytopathological Society, St. Paul, Minnesota. ISBN: 978-0-89054-448-8

Scott, S.W., M.T. Zimmerman and X. Ge (1998). The sequence of RNA 1 and RNA 2 of tobacco streak virus: additional evidence for the inclusion of alfalfa mosaic virus in the genus Ilarvirus. Archives of virology. 143: 1187-1198.

Tamer, A., M. Aydemir and A. Has (1997). Faunistic survey studies on harmful and beneficial insects on lucerne and sainfoin in Ankara and Konya provinces. Plant Protection Bulletin. 37(3-4): 125-161.https://dergipark.org.tr/tr/pub/bitkorb/issue/3660/48735

Tanasijevic, N., (1958). Zur Morphologie und Biologie des Luzernemarienkäfers *Subcoccinella vigintiquatuorpunctata* L. (Coleoptera: Coccinellidae). Beitragezur Entomologie.8: 23-78. DOI: 10.21248/contrib.entomol.8.1-2.23-78

Tucak, M., T. Čupić, D. Horvat, S. Popović, G. Krizmanić and M. Ravlić (2020).Variation of phytoestrogen content and major agronomic traits in alfalfa (*Medicago sativa* L.) populations. Agronomy. 10(1): 87, 1-11. DOI: 10.3390/agronomy10010087

Usta, M. and A. Güller (2020). Molecular Characterization of the Coat Protein Genome of Alfalfa Mosaic Virus (AMV) Isolates from Alfalfa in Van Province. J. the Institute of Science and Technology. 10(4): 2366-2377. DOI: 10.21597/jist.719099

Van Regenmortel, M.H.V. and L. Pinck (1981). "Alfalfa Mosaic Virus" Handbook of plant virus infections and comparative diagnosis, part II. Elsevier/ Nort Holland Biomedical Press; Amsterdam. 943 p.

Wang, Z., Q. Ke, M.D. Kim, S.H. Kim, C.Y. Ji, J.C. Jeong, H.S. Lee, W.S. Park, M.J. Ahn, H. Li, B. Xu, X. Deng, S.H. Lee, Y.P. Lim and S.S. Kwak (2015). Transgenic alfalfa plants expressing the sweet potato orange gene exhibit enhanced abiotic stress tolerance. Plos One. 10, p., e0126050. DOI: 10.1371/journal.pone.0126050

Wheeler, A.G. and T.J. Henry (1981). Seasonal history and habits of the European alfalfa beetle, *Subcoccinella vigintiquatuorpunctata* (L.) (Coleoptera: Coccinellidae). The Coleopterists Bulletin. 35: 197-203.

Wielkopolan, B., M. Jakubowska and A. Obrępalska-Stęplowska (2021). Beetles as plant pathogen vectors. Frontiers in Plant Science, 2241. DOI: 10.3389/fpls.2021.748093

Willsey, T., S. Chatterton and H. Cárcamo (2017). Interactions of root-feeding insects with fungal and oomycete plant pathogens. Front. Plant Sci. 8:1764. DOI: 10.3389/fpls.2017.01764

Xu, H. and J. Nie (2006). Identification, characterization, and molecular detection of Alfalfa mosaic virus in potato. Phytopathology. 96(11): 1237-1242. DOI: 10.1094/PHYTO-96-1237

Zschau, K. and C. Janke (1962). Samenubertragung des luzernemosaikvirusanluzerne. NachrBl. Dtsch. PFLSchDienst. 16: 94-96.