

OCCURRENCES OF WHEAT CURL MITE *ACERIA TOSICHELLA* KEIFER 1969 (ERIOPHYIDAE) AND THE ASSOCIATED VIRUSES, (WSMV, HPWMoV, TriMV) IN IRAQ

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ABSTRACT

This study was aimed to investigate the significant wheat yield losses in northern and central Iraq. The cause of these losses was examining the presence of wheat curl mite *Aceria tosichella* and three viruses vectored by this species – *Wheat streak mosaic virus* (WSMV), *High Plains wheat mosaic virus* (HPWMoV), and *Triticum mosaic virus* (TriMV). Mite specimens were collected from Erbil, Mosul, and Baghdad over three years (2020-2022) and identified using morphological characterization and DNA sequencing of the ITS region. Virus presence was determined using Double Antibody Sandwich-Enzyme-Linked Immunosorbent Assay (DAS-ELISA). Mites were identified conclusively as *A. tosichella*, providing the first record of this species in Iraq. All three viruses were found to be present, with WSMV having the largest presence as it was detected in 99.56%, then HPWMoV (79.5%) of mite colonies. This is the first record of HPWMoV anywhere in Eurasia besides Ukraine and its widespread presence in Iraq in all three sampled provinces. TriMV was detected only in Erbil. To date this virus has been recorded only in North America and its limited presence in Iraq. The confirmed presence of *A. tosichella* and three vectored-viruses in Iraq are all new records and critical information for reducing wheat yield losses in this country.

Key words: wheat streak mosaic virus, high plains wheat mosaic virus, triticum mosaic virus, eriophyid mite.

خلف وآخرون

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تواجد حَلْم التفاف اوراق الحنطة 1969 Keifer (*Aceria tosichella*) (Eriophyidae) والفايروسات المصاحبة له

(WSMV) و (HPWMoV) و (TriMV) في العراق

اليشيا ايغا تيم
باحث علمي

لينا ماريا أغيري روهاس
باحث علمي

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المستخلص

هدفت الدراسة الى التحقق من وجود انخفاض كبير في انتاج محصول الحنطة في شمال و وسط العراق. لوحظ ان السبب الاساسي في حدوث هذا الانخفاض وهو وجود حَلْم التفاف اوراق الحنطة *Aceria tosichella* وكذلك وجود ثلاثة فايروسات تنقل بواسطة هذا النوع من الحَلْم وهي *Wheat streak mosaic virus* (WSMV) و *High Plains wheat mosaic virus* (HPWMoV) و *Triticum mosaic virus* (TriMV). تم جمع عينات حَلْم التفاف اوراق الحنطة من ثلاث محافظات (أربيل ونيوى وبغداد) على مدى ثلاث سنوات متتالية (2020-2022)، اذ تم تحديد هذا النوع من الحَلْم بناءً على شكله الخارجي و تسلسل قواعده النيتروجينية للمنطقة الجينية ITS. كما وتم تحديد وجود كل من الفايروسات الثلاثة المنقولة بواسطة حَلْم التفاف اوراق الحنطة باستخدام تقنية DAS-ELISA. في هذه الدراسة، تم تسجيل حَلْم التفاف اوراق الحنطة *A. tosichella* وثلاثة فايروسات (WSMV و HPWMoV و TriMV) تنقل بواسطته لأول مرة في العراق. كما وبينت الدراسة ان WSMV هو الاكثر انتشاراً (99.56 %) و يليه HPWMoV (79.5%) في العينات التي تم جمعها. تجدر الإشارة الى ان تسجيل HPWMoV هو الاول من نوعه في المنطقة الواقعة ما بين قارتي اسيا و اوريا بالاضافة الى اوكرانيا. كما ان الدراسة سجلت TriMV في محافظة اربيل فقط، اذ تم تسجيل هذا النوع من الفايروس في الولايات المتحدة الامريكية فقط، حتى الان. يُعد الوجود المؤكد لحَلْم التفاف اوراق الحنطة والفايروسات الثلاثة المصاحبة له ذو اهمية كبيرة قد تساعد في الحد من انخفاض انتاج الحنطة في العراق.

الكلمات المفتاحية: الحَلْم الاريوفي، wheat streak mosaic virus، high plains wheat mosaic virus، triticum mosaic virus

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INTRODUCTION

Pests are responsible for most of the plant yield losses in agricultural crops (7, 8, 12, 13) due to agricultural practice (5, 6). Wheat curl mite *Aceria tosichella* Keifer is a significant crop pest that reduces wheat yields worldwide which can affect food security (37). The mite causes direct damage by feeding on leaf epidermal tissue, which causes rolling and trapping of the flag leaves. Wheat yield losses from *A. tosichella* feeding direct damage are estimated as high as 9- 30% (26, 27). Although most eriophyoid mite species are host-specific, feeding on only one or a few species within a single genus (38, 45). However, *A. tosichella* is regarded as an exception since over 100 species have been reported as hosts, including cultivated hosts such as sorghum, rye, barley, and maize and numerous wild types of grass (20, 27). *A. tosichella* can cause extensive economic indirect damage to cultivated crops by vectoring several plant viruses (34, 40, 52). Globally, *A. tosichella* transmits at least three devastating wheat viruses (14, 52). Of these viruses, the most widespread is *Wheat streak mosaic virus* (WSMV; genus *Tritimovirus*), which causes losses of 2.5 to 7%, although the severity of infection depends on the wheat cultivar and weather conditions (44, 52). The two other major viruses vectored by *A. tosichella* are *High Plains wheat mosaic virus* (HPWMOV, genus *Emaravirus*) and *Triticum mosaic virus* (TriMV; genus *Poacevirus*) (18, 19, 44); all three viruses cause symptoms such as severe yellowing, necrosis, and stunting, resulting in extensive wheat damage. Wheat curl mite is distributed mainly in wheat-producing regions in Europe and the Americas, although populations are presumed to occur throughout the Northern Hemisphere where cultivated or wild grains are grown. It also contributes to rapid dispersal among plants by wind (41). These factors contribute to uncertainty regarding the exact geographic distribution of *A. tosichella*. Although the species is thought to be widely distributed worldwide it has only been officially recorded in the Middle East in Jordan (46), Turkey (50), Syria (35, 39) and Iran (24, 53). In recent years, wheat yield reduction has been observed in the north and central regions of Iraq. Although it is likely that these losses are due

to damage caused by *A. tosichella*, this species has not yet been recorded in this country. The aim of this study was therefore to investigate the presence of *A. tosichella* as well as the viruses vectored by this species in Erbil, Mosul, and Baghdad. Since *A. tosichella* is one of several eriophyoid mite species known to occur on grass hosts and is morphologically similar to other mite species within the genus, including *A. tulipae*, correct identification essential. Since misidentification is common based on morphology alone, we used molecular diagnosis based on nucleotide sequences of the internal transcribed spacer (ITS) region, which is known to provide accurate results for species identification.

MATERIALS AND METHODS

Wheat curl mites were obtained from wheat heads, *Triticum aestivum* L. collected in three provinces in Iraq – Erbil, Mosul and Baghdad (Table 1). Mites were collected over a period of three years (2020-2023) from various locations each year. To provide an acceptable representation of mite populations, ten wheat head samples were randomly taken from each location. Five heads were selected arbitrarily to avoid bias. Ten female mites were transferred from each head and individually placed in a microcentrifuge 0.2 ml tube using a NASCO microscope at $\times 40$ magnification. Each microcentrifuge 0.2 ml tube was centrifuged at 13,000 rpm at 4°C for 45 sec. to allow the mite to be positioned in the bottom of the tube, then stored at -80°C. The remaining specimens were put in 70% alcohol and retained as vouchers at the Department of Plant Protection, College of Agricultural Engineering Science, University of Baghdad. Mites were identified morphologically according to shape and size following Orlob (40) at the Acarology lab, Department of Plant Protection, College of Agricultural Engineering Science, University of Baghdad. Five specimens were randomly selected for molecular identification. DNA extraction and amplification 600bp of the nuclear ribosomal internal transcribed spacer 1 (ITS1) region were followed (33). PCR amplicons were sequenced in both forward and reverse directions by Macrogen Inc. Geumchen, Seoul, South Korea. Only clear chromatographs were analyzed, ensuring that sequence annotations

and variations were not due to PCR or sequencing artifacts. Sequences were edited,

aligned, and analyzed using BioEdit Sequence Alignment Editor Software Version 7.1 (25).

Table 1. GPS coordinates for locations of *A. tosichella* samples collected from three provinces in Iraq - Erbil, Mosul, and Baghdad

Location (Iraq) Province	Specimen code	GPS Coordinate (Latitude, Longitude)	Collection date (mm.dd.yyyy)
Erbil	E1	36.135622, 43.735785	06.19.2020
	E2	36.153493, 43.751278	06.20.2020
	E3	36.168652, 43.928105	06.21.2020
	E4	36.148982, 43.948918	06.22.2020
	E5	36.133147, 43.733888	06.08.2021
	E6	36.151960, 43.749991	06.08.2021
	E7	36.170116, 43.929167	06.09.2021
	E8	36.127130, 43.954620	06.10.2021
	E9	36.135633, 43.735557	06.23.2022
	E10	36.133110, 43.733659	06.23.2022
	E11	36.168629, 43.928190	06.23.2022
	E12	36.170222, 43.929147	06.22.2022
Mosul	M1	36.287840, 43.155199	05.29.2020
	M2	36.273178, 43.178206	05.29.2020
	M3	36.274037, 43.190423	05.30.2020
	M4	36.256038, 43.204367	05.30.2020
	M5	36.275115, 43.184364	05.25.2021
	M6	36.285669, 43.154931	05.25.2021
	M7	36.270542, 43.190477	05.26.2021
	M8	36.252015, 43.204764	05.26.2021
	M9	36.247375, 43.203450	05.28.2022
	M10	36.243602, 43.204459	05.28.2022
	M11	36.239502, 43.215775	05.28.2022
	M12	36.241932, 43.212055	05.28.2022
Baghdad	B1	33.187880, 43.995996	03.26.2020
	B2	33.198490, 43.981982	03.26.2020
	B3	33.194907, 44.007192	03.26.2020
	B4	33.197573, 43.994950	03.26.2020
	B5	33.203257, 43.965159	04.18.2021
	B6	33.232598, 43.961876	04.18.2021
	B7	33.202296, 44.019155	04.18.2021
	B8	33.237516, 43.961157	04.18.2021
	B9	33.216327, 43.955117	04.10.2022
	B10	33.237974, 43.950900	04.10.2022
	B11	33.205699, 43.985458	04.10.2022
	B12	33.201550, 44.004081	04.10.2022

Observed variations in each sequence were numbered according to their corresponding position within the reference genome. Sequences were deposited in GenBank with the unique accession numbers OQ674818-OQ674822. Biotype assignments were assessed by comparing generated sequences with those of confirmed biotypes 1 and 2 from GenBank. Maximum likelihood trees were constructed in Geneious Prime 2021.0.3 using the RAxML plugin (48). Bootstrap support values were calculated based on 1,000 replicates. Viruses harbored in the mite were examined by maintaining mites on susceptible wheat plants and assessing viral presence in the plants. A mite colony from each location was established by transferring 250 mite individuals from each head/location to

susceptible Abu Ghraib wheat plants in POTGROND soil (Reef AL-Khadhraa CO., Iraq). Each mite colony was kept separately (4) in a 55×55×65 cm mite-proof mesh cage (Reef AL-Khadhraa CO., Iraq). Two wheat seeds were planted per pot and only one seedling was maintained after germination. Ten viruliferous females adult wheat curl mites from each mite colony were placed on five wheat plants on two-leaf stage in the second leaf whorl. Mites were left to feed for 21 days to allow plants to develop possible virus symptoms. Virus was detected in wheat leaf tissue 21 days post-infestation by enzyme-linked immuno-sorbent assay (ELISA). Three viruses, WSMV, HPWMoV, and TriMV, were screened using the double-antibody sandwich (DAS)- ELISA PathoScreen kit (Agdia Inc.,

Elkhart, IN) following the manufacturer's protocol. Absorbance at 405 nm was measured after 1 h, using a BioTek 800 TS absorbance reader (BioTek, Santa Clara California, USA), following the manufacturer's instructions. ELISA values were used to calculate an ELISA ratio (OD 405 nm value of the infected plant leaf/OD 405 nm value of healthy control leaf), where a high ratio (≤ 2.0) indicates disease presence and a low ratio (> 2.0) indicates absence (23). Data for virus infection were analyzed independently for each location/year. ELISA ratios for virus infection data were analyzed using one-way ANOVA PROC GLIMMIX (42).

RESULTS AND DISCUSSION

Mite identification: The identity of mites feeding on wheat in three provinces in Iraq was confirmed using DNA sequences of the ITS1 region from five individuals and comparing them to known sequences in the NCBI database. These results and phylogenetic trees (Fig. 1) showed that mites could be conclusively identified as *A. tosichella*. Although recent wheat yield losses in Iraq cannot categorically be attributed to this species symptoms observed in the field are similar to those caused by the mite and the viruses that it vectors. Wheat curl mite is distributed mainly in Europe and the Americas in areas where wheat is produced, although populations are presumed to occur throughout the Northern Hemisphere. Despite their presumably wide distribution, the species has only been officially recorded in the Middle East in Jordan (46), Turkey (50) and Iran (24, 53). This study is the first confirmed report of *A. tosichella* presence in Iraq. The dissemination pathways of wheat curl mite throughout the world are uncertain since its first record as *A. tulipae* in the United Kingdom in 1965 (21). The microscopic body size of the mites, which ranges from 190 – 220 μm in length (21), allows it to escape detection until transmitted viruses cause severe damage, resulting in unreliable reconstructions of colonization routes. Determining the invasion pathway(s) of the mite in Iraq, and its first occurrence in this country would therefore be speculative without further research. Results of DNA sequencing of mites collected in Iraq provided information on genetic lineages of *A.*

tosichella present in this country. The species is known to occur as a mixture of several genetic lineages. Two genetically distinct lineages, which can be discriminated using ITS sequence differences, are known to be wheat pests around the world. These lineages may differ both in their feeding response to different wheat varieties (28) and in their ability to transmit WSMV, HPWMoV and TriMV (36, 43). Sequence comparisons of individuals collected in Iraq showed that both lineages are present in this country (Fig 1). Due to small sample sizes the proportion of each lineage in Iraq were not measured. Future studies will examine how the two lineages vary in both space and time in Iraq, and if additional lineages are present. This information could improve *A. tosichella* management by allowing producers to select wheat varieties most resistant to damage by this mite. In addition, since wheat curl mite strains may differ in their ability to transmit viruses, more detailed information on lineages present in Iraq would allow the most effective virus vectors to be distinguished.

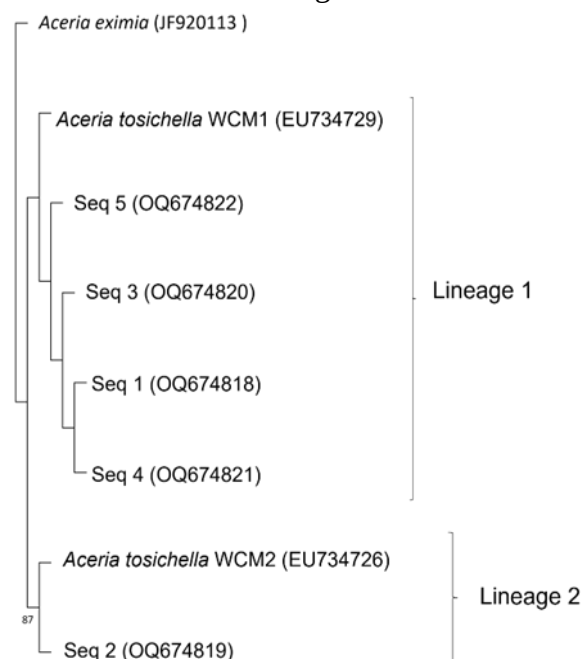


Fig. 1. Maximum likelihood tree showing the relationships among five *A. tosichella* individuals from Iraq (Seq 1-5) and representatives of the two main lineages worldwide (WCM1 and WCM2), with *A. eximia* as an outgroup. GenBank numbers are provided in brackets. Bootstrap values larger than 75% are shown.

Virus presence

The study investigated the presence of all three viruses in three provinces in Iraq. Results shown in tables 2- 4 indicate that all three viruses are present in Iraq. The most widely distributed virus in this study was WSMV, which was detected in 99.56% of mite colonies collected over three years and was harbored in all 36 sampled sites in Erbil, Mosul, and Baghdad, similar to Khalaf et al. (34). This geographic distribution was also true of HPWMoV, although this virus was less prevalent and only detected in 79.5% of sampled mite populations. The results in tables 2 and 3 highlight the presence of WSMV and HPWMoV, indicating a high virus titer (ELISA ratio) compared with the negative control. Populations from Mosul and Baghdad carried WSMV and HPWMoV as a coinfection. In comparison, TriMV, was present in Erbil only (Table 4), with low virus titer (low ELISA ratio) and was found in only 26.67% of mite colonies. Plant viruses have been detected on various hosts in Iraq (2, 3, 9), but only three viruses have been known to infect wheat and barley to date; these viruses include *Barley yellow dwarf virus-MAV* (10), *Barley yellow dwarf virus-PAV* (10) and *Cereal yellow dwarf virus-RPV* (22). *Wheat streak mosaic virus* has a host range that, in addition to wheat, includes barley, oats, corn (16) and several weed species. Its relatively broad host range has resulted in the spread of this virus in many countries around the world. In the Middle East, WSMV has been detected in Jordan, Turkey, Iran and Syria. It has been recorded in Turkey on wheat, barley, oats and triticale (17, 29), in Syria on wheat (35) and in Iran on weeds including *Digitaria sanguinalis* (hairy crabgrass) and *Echinochloa colonum* (jungle rice) (24, 32). Given the broad geographic range of WSMV its presence in

Iraq is not unexpected. However, the presence of HPWMoV was unexpected since the disease has only been recorded in the United States (30), Canada (1), Argentina (11, 51), Ukraine (47) and Australia (31). To date, the virus has not been recorded in anywhere in Eurasia besides Ukraine. Its widespread presence in Iraq, similar to that of WSMV, suggests that it has been established undetected for an extended period of time and raises the question of whether its presence extends beyond the borders of Iraq. The host range of HPWMoV includes cultivated crop plants such as oats, pearl millet, barley, rye and corn in addition to wheat and various weed species (1, 30, 49). Further research will clarify whether HPWMoV is present on additional hosts in Iraq. The presence of TriMV in Iraq was also unanticipated. The virus was first identified in 2006 and since then has only been recorded in the Great Plains region of the United States (19) and recently also in Canada (15). This is the first report of TriMV outside of the New World. Results from Iraq show that this virus was detected only in Erbil but was absent in Mosul and Baghdad. This limited distribution suggests a recent introduction of TriMV in Iraq. Additional research is necessary to further determine its prevalence in this country and economic losses incurred as a result of its presence. Wheat curl mite populations from Erbil were able to transmit all three viruses surveyed (WSMV, HPWMoV and TriMV) and results appear to suggest that mites are able to harbor three viruses simultaneously. The results of ELISA ratios (Table 5) for WSMV, HPWMoV and TriMV showed significant differences in susceptible wheat plants infested by mites from all studied locations,

Table 2. Presence/absence of *Wheat streak mosaic virus* (WSMV) indicated by ELISA absorbance values (405 nm) of leaves from Abu Ghraib wheat plants infected with wheat curl mite for 21d or left uninfected.

Province	WSMV Specimen code	ELISA value (absorbance) of infected plant										ELISA value (absorbance) of uninfected control plant			
		Rep 1	+ / -	Rep 2	+ / -	Rep 3	+ / -	Rep 4	+ / -	Rep 5	+ / -	Rep 1	+ / -	Rep 2	+ / -
Erbil	E1	2.623	+	2.347	+	2.577	+	2.714	+	2.699	+	0.012	-	0.054	-
	E2	2.651	+	2.798	+	2.689	+	2.509	+	2.591	+	0.057	-	0.049	-
	E3	2.711	+	2.333	+	2.794	+	2.207	+	2.455	+	0.057	-	0.085	-
	E4	2.506	+	2.788	+	2.751	+	2.713	+	2.709	+	0.074	-	0.063	-
	E5	2.656	+	2.235	+	2.413	+	2.248	+	2.742	+	0.088	-	0.071	-
	E6	2.517	+	2.690	+	2.239	+	2.498	+	2.679	+	0.079	-	0.028	-
	E7	2.148	+	2.199	+	2.337	+	2.459	+	2.701	+	0.074	-	0.054	-
	E8	2.543	+	2.639	+	2.551	+	2.163	+	2.319	+	0.100	-	0.081	-
	E9	2.914	+	2.492	+	2.738	+	2.717	+	2.543	+	0.098	-	0.069	-
	E10	2.791	+	2.677	+	2.712	+	1.051	-	2.687	+	0.100	-	0.089	-
	E11	2.326	+	2.723	+	2.160	+	2.301	+	2.390	+	0.097	-	0.111	-
	E12	2.508	+	2.560	+	2.676	+	2.656	+	2.431	+	0.091	-	0.101	-
Mosul	M1	2.611	+	2.336	+	2.455	+	2.609	+	2.713	+	0.098	-	0.085	-
	M2	2.594	+	2.003	+	2.106	+	2.347	+	2.741	+	0.088	-	0.079	-
	M3	2.194	+	2.711	+	2.302	+	2.111	+	2.123	+	0.077	-	0.083	-
	M4	2.327	+	2.610	+	2.714	+	2.131	+	2.766	+	0.096	-	0.079	-
	M5	2.001	+	2.138	+	2.692	+	2.105	+	2.100	+	0.098	-	0.089	-
	M6	2.200	+	2.701	+	2.199	+	2.400	+	2.401	+	0.042	-	0.055	-
	M7	2.520	+	2.413	+	2.146	+	2.345	+	2.421	+	0.049	-	0.044	-
	M8	2.234	+	2.211	+	2.299	+	2.209	+	2.235	+	0.059	-	0.121	-
	M9	2.679	+	2.012	+	2.219	+	2.409	+	2.394	+	0.075	-	0.083	-
	M10	2.293	+	2.422	+	2.138	+	2.309	+	2.513	+	0.090	-	0.103	-
	M11	2.120	+	2.199	+	2.297	+	2.329	+	2.401	+	0.088	-	0.085	-
	M12	2.199	+	2.509	+	2.611	+	2.222	+	2.419	+	0.091	-	0.109	-
Baghdad	B1	2.408	+	2.415	+	2.711	+	2.498	+	2.249	+	0.052	-	0.078	-
	B2	2.248	+	2.157	+	2.409	+	2.492	+	2.597	+	0.126	-	0.158	-
	B3	0.124	-	2.300	+	2.710	+	2.419	+	2.510	+	0.058	-	0.064	-

B4	2.109	+	2.298	+	2.399	+	2.577	+	2.361	+	0.094	-	0.056	-
B5	2.277	+	2.199	+	2.317	+	2.548	+	0.219	-	0.083	-	0.089	-
B6	2.144	+	2.113	+	2.316	+	2.198	+	2.219	+	0.155	-	0.077	-
B7	2.392	+	2.313	+	0.122	-	2.316	+	2.214	+	0.087	-	0.035	-
B8	0.155	-	2.233	+	0.019	-	2.407	+	2.711	+	0.086	-	0.088	-
B9	2.465	+	2.338	+	2.144	+	2.622	+	2.246	+	0.100	-	0.051	-
B10	2.503	+	2.263	+	2.330	+	2.277	+	2.191	+	0.165	-	0.059	-
B11	2.345	+	0.109	-	2.311	+	2.209	+	0.241	-	0.091	-	0.122	-
B12	2.448	+	2.310	+	2.203	+	2.289	+	2.511	+	0.098	-	0.126	-

Table 3. Presence/absence of *High Plains wheat mosaic virus* indicated by ELISA absorbance values (405 nm) of leaves from Abu Ghraib wheat plants infected with wheat curl mite for 21d or left uninfected

Province	HPWMOV Specimen code	ELISA value (absorbance) of infected plant										ELISA value (absorbance) of uninfected control plant			
		Rep 1	+ / -	Rep 2	+ / -	Rep 3	+ / -	Rep 4	+ / -	Rep 5	+ / -	Rep 1	+ / -	Rep 2	+ / -
Erbil	E1	1.203	-	2.123	+	0.215	-	2.147	+	2.192	+	0.011	-	0.041	-
	E2	2.157	+	1.197	-	2.028	+	2.099	+	2.187	+	0.019	-	0.022	-
	E3	1.111	-	2.098	+	0.401	-	0.271	-	2.248	+	0.090	-	0.084	-
	E4	2.028	+	2.159	+	2.198	+	2.318	+	2.257	+	0.049	-	0.038	-
	E5	2.008	+	2.316	+	2.193	+	2.152	+	2.279	+	0.029	-	0.072	-
	E6	2.199	+	2.201	+	1.282	-	2.317	+	2.258	+	0.071	-	0.040	-
	E7	2.185	+	2.107	+	2.313	+	2.421	+	2.210	+	0.081	-	0.062	-
	E8	2.482	+	2.311	+	2.145	+	2.128	+	2.190	+	0.142	-	0.181	-
	E9	2.140	+	2.201	+	2.302	+	2.173	+	1.300	-	0.082	-	0.041	-
	E10	2.128	+	2.159	+	2.102	+	1.198	+	2.541	+	0.111	-	0.027	-
	E11	1.290	-	2.203	+	2.171	+	2.101	+	2.110	+	0.049	-	0.094	-
	E12	2.271	+	2.287	+	0.753	-	2.214	+	2.187	+	0.083	-	0.191	-
Mosul	M1	2.329	+	2.259	+	1.582	+	2.201	+	2.139	+	0.099	-	0.097	-
	M2	2.137	+	2.199	+	2.288	+	1.300	-	2.179	+	0.028	-	0.026	-
	M3	2.254	+	2.233	+	2.285	+	1.159	-	2.183	+	0.068	-	0.083	-
	M4	2.111	+	2.197	+	2.117	+	1.099	-	2.117	+	0.065	-	0.069	-
	M5	2.154	+	2.219	+	2.209	+	2.182	+	2.188	+	0.072	-	0.071	-
	M6	2.219	+	2.212	+	2.121	+	2.167	+	2.301	+	0.095	-	0.048	-

Baghdad	M7	2.208	+	2.103	+	2.133	+	2.199	+	2.122	+	0.057	-	0.065	-
	M8	2.254	+	1.208	-	2.211	+	2.219	+	1.215	-	0.087	-	0.070	-
	M9	2.258	+	2.159	+	2.272	+	2.222	+	2.243	+	0.051	-	0.053	-
	M10	2.211	+	1.292	-	2.219	+	1.199	-	1.193	-	0.099	-	0.113	-
	M11	2.213	+	2.201	+	2.200	+	2.297	+	2.193	+	0.079	-	0.081	-
	M12	1.257	-	2.193	+	2.218	+	2.197	+	1.229	-	0.108	-	0.129	-
	B1	2.157	+	2.229	+	2.171	+	1.219	-	2.251	+	0.051	-	0.059	-
	B2	2.248	+	2.122	+	2.277	+	2.208	+	2.237	+	0.126	-	0.158	-
	B3	0.124	-	2.300	+	2.710	+	2.419	+	2.510	+	0.109	-	0.104	-
	B4	2.213	+	1.218	-	0.109	-	2.178	+	2.169	+	0.090	-	0.095	-
	B5	2.179	+	2.201	+	0.137	-	2.140	+	2.209	+	0.081	-	0.080	-
	B6	2.154	+	0.193	-	2.165	+	2.188	+	2.222	+	0.100	-	0.092	-
	B7	2.231	+	2.103	+	0.199	-	0.106	-	2.104	+	0.071	-	0.075	-
	B8	0.191	-	1.215	-	2.189	+	2.207	+	0.191	-	0.086	-	0.091	-
	B9	2.192	+	2.133	+	0.214	-	2.189	+	2.213	+	0.111	-	0.091	-
	B10	2.258	+	2.236	+	1.380	-	1.197	-	2.209	+	0.152	-	0.141	-
	B11	2.219	+	2.202	+	2.108	+	2.122	+	2.214	+	0.082	-	0.089	-
	B12	1.129	-	2.241	+	2.119	+	1.211	-	0.159	-	0.091	-	0.088	-

Table 4. Presence/absence of *Triticum mosaic virus* indicated by ELISA absorbance values (405 nm) of leaves from Abu Ghraib wheat plants infected with wheat curl mite for 21d or left uninfected

Province	TriMV Specimen code	ELISA value (absorbance) of infected plant										ELISA value (absorbance) of uninfected control plant			
		Rep 1	+ / -	Rep 2	+ / -	Rep 3	+ / -	Rep 4	+ / -	Rep 5	+ / -	Rep 1	+ / -	Rep 2	+ / -
Erbil	E1	0.415	+	1.215	-	2.35	+	1.151	-	2.327	+	0.020	-	0.012	-
	E2	2.393	+	2.213	+	2.136	+	2.363	+	2.229	+	0.099	-	0.091	-
	E3	1.237	-	2.311	+	2.429	+	1.410	-	1.342	-	0.051	-	0.018	-
	E4	2.298	+	2.299	+	2.319	+	2.309	+	2.324	+	0.098	-	0.088	-
	E5	2.208	+	2.206	+	2.325	+	2.219	+	2.189	+	0.058	-	0.069	-
	E6	1.218	-	0.299	-	2.312	+	2.382	+	2.392	+	0.015	-	0.018	-
	E7	2.219	+	2.251	+	2.231	+	2.352	+	2.382	+	0.096	-	0.092	-
	E8	1.358	-	2.355	+	2.315	+	2.099	+	2.329	+	0.128	-	0.136	-
	E9	2.154	+	2.152	+	1.200	-	2.189	+	2.129	+	0.058	-	0.049	-

Mosul	E10	2.188	+	2.179	+	2.175	+	1.181	+	2.201	+	0.089	-	0.079	-
	E11	2.199	+	2.220	+	2.183	+	1.169	-	1.191	-	0.054	-	0.063	-
	E12	2.209	+	2.219	+	2.218	+	2.155	+	2.198	+	0.072	-	0.094	-
	M1	0.291	-	0.239	-	0.159	-	0.117	-	0.211	-	0.029	-	0.033	-
	M2	0.213	-	0.220	-	0.229	-	0.212	-	0.221	-	0.101	-	0.100	-
	M3	0.297	-	0.313	-	0.297	-	0.401	-	0.390	-	0.093	-	0.103	-
	M4	0.128	-	0.193	-	0.127	-	0.155	-	0.199	-	0.075	-	0.079	-
	M5	0.251	-	0.321	-	0.137	-	0.321	-	0.244	-	0.072	-	0.071	-
	M6	0.219	-	0.212	-	0.121	-	0.167	-	0.301	-	0.095	-	0.048	-
	M7	0.208	-	0.103	-	0.422	-	0.250	-	0.203	-	0.053	-	0.055	-
	M8	0.150	-	0.318	-	0.201	-	0.190	-	0.105	-	0.093	-	0.099	-
	M9	0.618	-	0.357	-	0.901	-	0.809	-	0.643	-	0.049	-	0.028	-
Baghdad	M10	0.893	-	0.732	-	0.587	-	0.499	-	0.790	-	0.082	-	0.109	-
	M11	0.978	-	0.900	-	0.901	-	0.891	-	0.722	-	0.098	-	0.079	-
	M12	0.222	-	0.612	-	0.938	-	0.800	-	0.680	-	0.100	-	0.111	-
	B1	0.673	-	0.913	-	0.488	-	0.519	-	0.699	-	0.019	-	0.025	-
	B2	0.431	-	0.581	-	0.639	-	0.933	-	0.837	-	0.110	-	0.091	-
	B3	0.666	-	0.509	-	0.565	-	0.499	-	0.588	-	0.091	-	0.103	-
	B4	0.913	-	0.977	-	0.899	-	0.996	-	0.890	-	0.098	-	0.097	-
	B5	0.871	-	0.738	-	0.753	-	0.709	-	0.892	-	0.088	-	0.087	-
	B6	0.563	-	0.800	-	0.933	-	0.816	-	0.799	-	0.101	-	0.096	-
	B7	0.519	-	0.683	-	0.700	-	0.711	-	0.689	-	0.064	-	0.071	-
	B8	0.633	-	0.738	-	0.801	-	0.771	-	0.753	-	0.081	-	0.089	-
	B9	0.900	-	0.883	-	0.769	-	0.811	-	0.779	-	0.080	-	0.098	-
	B10	0.629	-	0.760	-	0.809	-	0.703	-	0.849	-	0.055	-	0.068	-
	B11	0.537	-	0.680	-	0.801	-	0.769	-	0.802	-	0.012	-	0.021	-
	B12	0.609	-	0.718	-	0.688	-	0.721	-	0.806	-	0.080	-	0.071	-

Table 5. Mean $\pm$ CI ELISA ratio* for three viruses (WSMV, HPWMoV, and TriMV) harbored in *Aceria tosichella* individuals from Erbil, Mosul, and Baghdad after three weeks of feeding on susceptible Abu Ghraib wheat plants

Province	Year	Specimen Code**	WSMV	Mean $\pm$ (CI) HPWMoV	TriMV
Erbil	2020	E1	78.54 $\pm$ (72.68-84.10) b	60.61 $\pm$ (58.28-63.11) c	93.22 $\pm$ (89.26-96.75) a
		E2	49.95 $\pm$ (46.92-52.07) b	94.32 $\pm$ (91.03-98.51) a	23.86 $\pm$ (22.41-24.57) c
		E3	35.21 $\pm$ (33.08-36.63) b	14.08 $\pm$ (13.87-14.33) c	50.60 $\pm$ (48.51-53.08) a
		E4	39.32 $\pm$ (37.76-42.96) b	50.39 $\pm$ (52.31-54.68) a	24.83 $\pm$ (23.61-25.06) c
	2021	E5	30.92 $\pm$ (28.55-32.02) b	43.35 $\pm$ (41.64-45.86) a	35.11 $\pm$ (34.75-36.25) b
		E6	47.18 $\pm$ (45.23-51.63) b	36.96 $\pm$ (34.71-38.76) c	104.28 $\pm$ (95.35-108.36) a
		E7	37.01 $\pm$ (35.83-39.86) a	31.43 $\pm$ (30.66-32.08) b	24.32 $\pm$ (23.71-25.88) c
		E8	26.99 $\pm$ (24.56-28.77) a	13.94 $\pm$ (13.26-14.84) b	15.84 $\pm$ (15.06-16.82) b
	2022	E9	32.10 $\pm$ (30.88-33.47) b	32.89 $\pm$ (30.81-35.16) a	36.72 $\pm$ (34.56-38.70) a
		E10	25.22 $\pm$ (23.09 -27.48) ab	29.35 $\pm$ (28.71-31.42) a	23.63 $\pm$ (22.52-24.19) b
		E11	22.88 $\pm$ (20.76-24.57) b	27.62 $\pm$ (26.42-28.94) ab	30.64 $\pm$ (28.25-32.17) a
		E12	26.73 $\pm$ (24.81-28.69) a	14.18 $\pm$ (13.82-15.05) b	26.50 $\pm$ (25.85-27.04) a
Mosul	2020	M1	27.81 $\pm$ (25.44-29.81) a	21.45 $\pm$ (20.97-22.58) b	6.56 $\pm$ (6.23-6.87) c
		M2	28.24 $\pm$ (26.51-31.08) b	74.84 $\pm$ (72.19-75.24) a	2.18 $\pm$ (2.04-2.33) c
		M3	28.60 $\pm$ (26.35-30.65) a	26.79 $\pm$ (26.21-27.07) a	3.46 $\pm$ (3.17-3.77) b
		M4	28.68 $\pm$ (25.61-30.97) a	28.78 $\pm$ (26.69-30.12) a	2.08 $\pm$ (1.89-2.27) b
	2021	M5	23.60 $\pm$ (21.46-25.04) b	30.63 $\pm$ (29.42-31.28) a	3.56 $\pm$ (3.16-3.82) c
		M6	49.07 $\pm$ (47.61-52.33) a	30.82 $\pm$ (30.15-32.62) b	2.85 $\pm$ (2.61-3.09) c
		M7	50.94 $\pm$ (48.71-53.46) a	35.29 $\pm$ (33.47-37.21) b	4.39 $\pm$ (4.05-4.75) c
		M8	24.86 $\pm$ (23.67-26.02) a	23.20 $\pm$ (22.74-24.66) a	2.01 $\pm$ (1.92-2.31) b
	2022	M9	29.65 $\pm$ (27.55-31.15) b	42.90 $\pm$ (42.37-43.09) a	17.28 $\pm$ (16.85-17.79) c
		M10	24.19 $\pm$ (22.67-25.98) a	15.31 $\pm$ (14.89-16.02) b	7.33 $\pm$ (6.87-7.74) c
		M11	26.23 $\pm$ (28.77-31.17) a	27.76 $\pm$ (26.54-28.66) a	9.92 $\pm$ (9.44-10.27) b
		M12	23.92 $\pm$ (21.56-24.09) a	15.34 $\pm$ (14.87-15.92) b	6.16 $\pm$ (5.94-6.42) c
Baghdad	2020	B1	37.78 $\pm$ (35.44-39.21) a	36.46 $\pm$ (34.73-38.51) a	29.92 $\pm$ (28.66-29.71) b
		B2	16.76 $\pm$ (16.20-17.31) a	15.62 $\pm$ (15.26-16.32) a	6.80 $\pm$ (6.37-7.41) b
		B3	32.99 $\pm$ (30.85-34.56) a	18.89 $\pm$ (17.86-19.20) b	5.83 $\pm$ (5.40-6.33) c
		B4	31.32 $\pm$ (30.25-32.54) a	17.05 (16.33-18.54) b	9.59 $\pm$ (9.23-10.45) c
	2021	B5	22.23 $\pm$ (21.56-23.72) a	22.03 $\pm$ (21.69-22.75) a	9.05 $\pm$ (8.61-9.57) b
		B6	18.94 $\pm$ (18.21-19.33) a	18.59 $\pm$ (18.21-19.33) a	7.94 $\pm$ (7.56-8.40) b
		B7	30.68 $\pm$ (28.31-32.47) a	18.47 $\pm$ (18.11-19.07) b	9.78 $\pm$ (9.24-10.17) c
		B8	17.29 $\pm$ (16.84-17.79) a	13.54 (13.04-14.86) a	8.69 $\pm$ (8.44-9.22) b
	2022	B9	31.29 $\pm$ (30.55-31.63) a	17.70 (16.54-18.37) b	9.31 $\pm$ (9.04-9.59) c
		B10	20.65 $\pm$ (20.13-21.09) a	12.66 (12.19-13.44) b	12.19 $\pm$ (11.82-12.63) b
		B11	13.55 $\pm$ (13.20-13.97) c	25.41 $\pm$ (24.73-26.22) b	43.50 $\pm$ (41.67-45.02) a
		B12	21.00 $\pm$ (20.63-21.85) a	15.32 (14.75-16.45) a	9.38 $\pm$ (9.13-9.75) b

Means with different letters in same row differed significantly * ($P \leq 0.05$). *ELISA ratio= OD405 value of infected leaf/OD405 value of healthy uninfected leaf. ** Specimen Code E= Erbil, M= Mosul, and B= Baghdad

except for two locations in Erbil (E10 and E11) where all three viruses were present at the same level. ELISA ratios of WSMV were significantly greater than those of HPWMoV and TriMV in 12 mite populations (E7, E8, M1, M6, M7, M10, M12, B3, B4, B7, B9, and B10). ELISA ratios of HPWMoV were significantly greater than those of WSMV and TriMV in 6 mite populations (E2, E4, E5, M2, M5, and M9), while TriMV ELISA ratios were significantly greater than WSMV and HPWMoV ELISA ratios in only 4 mite populations (E1, E3, E6, and B11). Our results provide evidence that the *A. tosichella* can harbor more than two viruses at the same time, although they may be present at low levels.

CONCLUSION: The results of this study showed that *A. tosichella*, as well as three

viruses vectored by this mite, are all present in Iraq, which are new records for each of the four species. This is also the first record of HPWMoV in the Middle East and the first record of TriMV outside of North America. All three viruses were able to occur as coinfections. This information is valuable for controlling populations of the mite and its associated viruses in Iraq, and for limiting further spread of the viruses in the Middle East and beyond.

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