OUTBREAK OF TOBAMOVIRUSES AND POTEXVIRUSES ASSOCIATED WITH DISEASE EPIDEMICS IN TOMATO PRODUCTION AREA OF IRAQ

Hasaneen K. Obaid[1] **Mustafa Adhab[1]**
Researcher **Assist. Prof.**
Department of Plant Protection, Coll. of Agric. Engi. Sci., University of Baghdad.
Email: mustafa.a@coagri.uobaghdad.edu.iq

ABSTRACT

This study was initiated to examine the tomato-infecting viruses belonging to the *Tobamovirus* and *Potexvirus* genera in Iraq. Field observations and surveys were carried out for three successive cropping seasons (2020/21 to 2022/23) in selected tomato production areas. The purpose was to identify the main viruses associated with tomato epidemics and assess the impact of different tomato cultivars on disease occurrence. A total of 700 tomato leaf samples were collected from seven governorates (Baghdad, Diyala, Babylon, Najaf, Kerbala, Nasiriya, and Basrah) and tested using pathogen-specific immunostrip kits. The survey showed a presence of *Tomato brown rugose fruit virus* (ToBRFV), *Tobacco mosaic virus* (TMV), *Pepper mild mottle virus* (PMMoV), *Cymbidium mosaic virus* (CymMV), *Odontoglossum ringspot virus* (ORSV), *Cucumber green mottle mosaic virus* (CGMMV), *Pepino mosaic virus* (PepMV) and *Potato virus X* (PVX) in tomato fields in Iraq. ToBRFV secured the highest relative incidence in tomato fields (7 governorates) followed by PepMV and CymMV and PMMoV (6 out of 7 governorates) and CGMMV, TMV (5 governorates), and PVX (3 governorates). The least was ORSV (only in Basrah). To our knowledge, this is the first comprehensive survey investigating *Tobamovirus* and *Potexvirus* on tomato fields in Iraq and the first report of ToBRFV, PMMoV, CymMV, ORSV, CGMMV and PepMV infecting tomato crops in Iraq.

Keywords: ToBRFV; PepMV; CymMV; ORSV; CGMMV; PepMV.
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تفشي فايروسات مجموعتي فايروس موزائيك التبغ وأكس البطاطا المرافق للوباء في مناطق إنتاج الطماطة في العراق

حسنين خلف عبيد **مصطفى علي عذاب**
باحث علمي **استاذ مساعد**
قسم وقاية النبات، كلية علوم الهندسة الزراعية ، جامعة بغداد ، بغداد ، العراق

المستخلص

اجريت هذه الدراسة للتحري عن الفيروسات التي تصيب الطماطة والتي تعود إلى جنس *Tobamovirus* و *Potexvirus* في العراق. أُجريت عمليات رصد واستقصاء ميدانية خلال ثلاثة مواسم زراعية متعاقبة (21/2020 إلى 23/2022) في مناطق إنتاج الطماطة في العراق. كان الغرض من الدراسة هو تحديد الفيروسات الرئيسية وتقييم أثرها في اصناف الطماطة المختلفة. تم جمع 700 عينة من نباتات الطماطة من سبع محافظات (بغداد، ديالى، بابل، النجف، كربلاء، الناصرية، والبصرة) وسط وجنوب العراق. أظهرت الاختبارات الحقلية التي أجريت باستخدام الاشرطة المناعية ان فيروس تنخر الثمار البني للطماطة (ToBRFV) وفيروس موزائيك التبغ (TMV) وفيروس تبرقش الفلفل المعتدل (PMMOV) وفيروس موزائيك الزورقان (CymMV) وفيروس التبقع الحلقي للسانيات (ORSV) وفيروس موزائيك تبرقش الخيار الأخضر (CGMMV) وفيروس موزائيك الببينو(PepMV) وفيروس البطاطا أكس (PVX) تصيب حقول الطماطة في العراق. وتم رصد أعلى نسبة للإصابة بفايروس ToBRFV (جميع المحافظات السبعة) بفيروسات PepMV وCymMV وPMMoV (6 من أصل 7 محافظات) يليها CGMMV و TMV (في 5 محافظات) و PVX (3 محافظات)، في حين أقل الفيروسات انتشارا كان ORSV (فقط في البصرة). على حد علمنا، هذا هو اول مسح شامل لحقول الطماطة في العراق وأول تسجيل لفايروسات ToBRFV وPMMoV وCymMV وORSV وCGMMV وPepMV تصيب محصول الطماطة في العراق.

الكلمات الرئيسية: ToBRFV; PepMV; CymMV; ORSV; CGMMV; PepMV
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INTRODUCTION

Tomato (*Solanum lycopersicum* L.; Family: Solanaceae) is one of the major produced vegetables globally including Iraq (2, 16). Tomatoes are susceptible to a wide range of biotic and abiotic stresses (8, 12, 14), including at least 140 different viruses (23). Several viruses were reported to infect tomato plants in Iraq. Those are *Potato virus X* (PVX; genus: *Potexvirus*), *Tomato aspermy virus* and *Cucumber mosaic virus* (TAV and CMV; genus: *Cucumovirus*), *Tomato bushy stunt virus* and *Grapevine Algerian latent virus* (TBSV and GALV; genus: *Tombusvirus*), *Tobacco mosaic virus* and *Tomato mosaic virus* (TMV and ToMV; genus: *Tobamovirus*), *Tomato spotted wilt virus* (TSWV; genus: *Tospovirus*), *Tomato yellow leaf curl virus* and *Tomato leaf curl Palampur virus* (TYLCV and ToLCPMV; genus: *Begomovirus*) (3). The mechanically transmitted viruses are highly detrimental to tomato plants, causing significant damage. The infection with ToMV resulted in a significant decrease in the fresh (89%) and dry (91%) weights of tomato plants. Additionally, it was demonstrated that ToMV resulted in a significant decrease in both the length of the shoot (68.7%) and root systems (62.8%), as compared to healthy plants (10). The virus also caused a reduction in the chlorophyll content, up to 42%. Due to virus infections, losses are not restricted to tomato crops (19, 20) as viruses can limit agriculture in Iraq. Sap transmission of plant viruses involves the transfer of viruses from diseased to healthy plants by physical mechanisms, including contaminated tools, hands, or equipment. This mode of transmission is significant due to its ability to facilitate rapid dissemination, effortless transmission, extensive distribution, and creation of virus management complexities (3).The entry of viruses to a new geographic location as a source of emergence is frequently associated with continuing global change. The widespread expansion of international trade and the development of modern transportation infrastructure enable the massive spread of disease-causing organisms over long distances (30). The swift worldwide spread of ToBRFV throughout many continents serves as a prime example of these trends (25, 26). Another example of this occurrence is the inquiry conducted by (18), which revealed the existence of *Tomato yellow leaf curl virus* (TYLCV) in imported packaged tomatoes in Sweden and Estonia. Climate change can be a contributing element that affects the frequency and transmission of viruses (17). Due to the increasing average yearly temperatures, dramatic changes were already observed in the spread of disease-carrying organisms. Therefore, it is reasonable to anticipate that this will result in fresh prospects for the virus dissemination. Even though many studies have explored the resistance against viruses and suggested solutions (4, 5, 6). Mechanically transmitted viruses are still a serious threat to crops worldwide. They cost the agriculture business hundreds of millions of dollars. Iraq is experiencing a rising trend in the emergence of unidentified viruses. A higher prevalence of virus-like symptoms is reported on tomato crop. Against this background, this study aimed to investigate the prevalence and distribution of mechanically transmitted viruses in the national main tomato industry, the middle and south of Iraq.

MATERIALS AND METHODS

Experiment setting and sampling: During the 2020 and 2023 growing seasons, tomato farms were observed and surveyed for mechanically transmitted viruses. Tomato farms located throughout the middle and south of Iraq's seven governorates (Baghdad, Diyala, Babylon, Najaf, Karbala, Nasiriya and Basrah) were inspected. The selection of farms was based on virus infection reported by farmers and agricultural professionals in the seven governorates. Two or three new emerging leaves were collected from symptomatic and symptomless tomato plants. The locations of the farms surveyed and the percentage of infestation were recorded at each field and tunnel site to generate the distribution GPS accurate locations. A total of 100 sites and 700 tomato samples were assessed for the presence of eight mechanically transmitted plant viruses. The following disease index was used: low (1-20% of samples detected positive to at least one virus), medium (21-50%) or high (more than 50% of samples detected positive). A total of 100 samples per governorate was used in the screening process.

Serological assay and data collection

Field testing using Pathogen Immuno Strip Test specific kit (Agdia, USA) targeting *Tomato brown rugose fruit virus* (ToBRFV), *Tobacco mosaic virus* (TMV), *Pepper mild mottle virus* (PMMoV), *Cymbidium mosaic virus* (CymMV), *Odontoglossum ringspot virus* (ORSV), *Cucumber green mottle mosaic virus* (CGMMV), *Pepino mosaic virus* (PepMV) and *Potato virus X* (PVX) was performed on tomato plants. Leaf samples from each plant consisted of 1 cm diameter leaf discs, collected on the apical leaf (one disc per leaflet). Sap was tested using ImmunoStrip tests provided by Agdia Inc. (Elkhart, IN, USA) following manufacturing protocol as follows: A specimen extracted from plant tissue under test was placed in the Agdia sample extracting bag that was filled with 3 mL of extraction buffer. To achieve the appropriate 1:20 dilution, 0.15 g of tissue was used, which is approximately equivalent to an area of 1 square inch. To ensure proper hygiene, the cutting equipment was replaced for each sample or sterilized with 10% bleach solution. The sample was placed within the mesh linings located toward the lower part of the sample extraction bag. The sample was completely broken down using a blunt instrument like a pen or marker for homogenizing the sample. The extracted solution was allowed to rest undisturbed for a duration of 3 minutes before inserting the ImmunoStrip. The sample end of the ImmunoStrip was securely placed into the channel section of the bag (without mesh) until it was fully immersed in the extract, up to the white line located about ¼ inch from the bottom. Any contact between the ImmunoStrip and froth or bubbles (if they are present) was avoided. The bag was positioned vertically in a box. The ImmunoStrip test is kept in the sample extract for a duration of 30 minutes in a cool box. The ImmunoStrip was removed from the extract (sample) and the result was analyzed. A negative outcome is indicated when just the control line is visible. If the control line is observable and the test line is also detectable with any level of pink/purple coloration, it signifies the existence of the target pathogen. The presence of a control line ensures that the test is functioning correctly. An invalid test occurs if the control line is not there, regardless of whether a test line is visible.

Locations and tomato cultivars

Locations and tomato cultivars tested were recorded. The locations targeted in this study included the major tomato cultivation governorates in the middle and south of Iraq. These governorates and their GPS coordinates are Baghdad (Abu Ghraib [33.2976° N, 44.0818° E], Radhwaniyah [33.1853° N, 44.2674° E], Yousfiyah [33.0791° N, 44.2520° E], Latifiyah [32.9850° N, 44.3568° E], and research station in Jaderiyah [33.2796° N, 44.3851° E]), Diyala (Khan Bani Saad [33.5701° N, 44.5412° E], and Baquba [33.7517° N, 44.6081° E]), Babylon (Jbala farms and nurseries in Hilla [32.4736° N, 44.4252° E]), Karbala [32.6027° N, 44.0197° E], Najaf (Khan Al-Nus, and other desert tomato production locations [32.0107° N, 44.3265° E]), Naseriyah (Al-Gharraf [31.2985° N, 46.2427° E], nurseries in Naseriyah [31.0510° N, 46.2569° E]), and Basrah (Um Aneij [30.1672° N, 46.9805° E], Safwan [30.1097° N, 47.7194°E], and Haddamah – the oldest tomato cultivation area in Iraq [30.0398° N, 47.9268° E]). The tomato cultivars tested in this study were Latevia, Madina, Raoa'a, Mercury, Bashira, Basheer, Bushra, Coral, Jandar, Oscar, Gxara, Ibrahimi, Aseel, Ostora, Sultan, Yasmeen, Salima and some unknown cultivars (the farmer does not know the name). The cultivars were distributed in different locations and one cultivar may be found in more than one governorate. The percentage of infection was assessed following the formula:

Infection% = (Number of samples tested positive/total number of samples) X100

RESULTS AND DISCUSSION

Validation of sampling and serological test

The tomato open fields and tunnels selected for field observation and sample collection were located in regions of Iraq following intensive tomato cultivation, including Baghdad, Diyala, Babylon, Najaf, Karbala, Nasiriya and Basrah. The study included farms varied in tomato growing history and conditions, including old (over 100 years old) in Basrah, middle-aged farms (15–30 years old) in all the seven governorates, and some

newly established ones (1 to 10 years old) in Baghdad, Diyala, and Karbala. The tomato plants in each field were initially monitored through direct observation of the development of symptoms like those caused by viruses. The majority of plants in the assessed fields displayed no discernible signs indicative of viral infections, and samples were gathered randomly. Nevertheless, in certain tomato fields investigated, occasional instances of characteristic virus symptoms, such as chlorotic, stunting, mosaic, and mottle were identified on tomato leaves. Early observation of chlorotic and yellow blotches, followed by brown spots, and rugose wrinkles and lines appearing on tomato fruits was made in fields located in Diyala (Figure 1). These symptoms were found distinctive to ToBRFV. Different types of chlorosis, distortion, and stunting were observed on the leaves of the different tomato cultivars 'Latevia', 'Madina', 'Raoa'a', 'Mercury' in farms located in Baghdad, Najaf, Karbala, Babylon, Nasiriyah and Basrah governorates. The symptoms were observed in plants that were detected positive to TMV, ToBRFV, PMMoV, CymMV, ORSV, PVX, CGMMV, and PepMV, as confirmed by serological testing (table 1). Commonly, some virus-infected plants were symptomless. A single ToBRFV infection on tomato cultivar 'Ibrahimi' exhibited symptoms of black dots and blotches on tomato fruits was detected in fields at Basrah (Figure 1). Our data also indicated that the infection of tomato plants with ToBRFV and CymMV caused the tomato fruit ripening to be delayed 5 days due to infection. The presence of ToBRFV infection is linked to necrosis of leaves in seedlings and mosaic patterns with leaf distortion in older plants. This infection results in minor foliar symptoms and the development of brown rugosities on fruits. In their study, Luria et al. (26) documented the presence of both mild and severe mosaic variants of ToBRFV on leaves. They also observed occasional leaf narrowing and the development of yellow spots on the fruits of each affected plant.

Mechanically transmitted viruses infecting tomato fields and their effect on tomato production in Iraq: Yellow and green mosaic and mottling were frequently noticed on tomatoes in all tomato-producing zones. These symptoms are indicative of TMV and CymMV infections (Figure 2). We verified the presence of the viral infection by conducting immunostrip testing on-site. Tobamoviruses are spread through mechanical means and by the bumblebee (24). The primary culprits responsible for tomato plant issues were firstly ToMV and TMV. The utilization of resistance genes Tm-2 and Tm-2^2 in the development of tobamovirus-resistant cultivars played a significant role in managing this viral disease (1, 22). An emerging Jordanian strain of ToBRFV has recently overcome resistance and caused significant damage to the global tomato crop (26). The other group we investigated is potexvirus, a previously not common on tomato in Iraq. PepMV is a member of the *Potexvirus* genus in the *Alphaflexiviridae* family (31). Tomato fields in Iraq have shown typical signs produced by this virus, such as yellow mosaic, leaf deformation, development of yellow speckles and spots on ripe fruits associated with bubbling of the leaf surface. While certain genotypes of PepMV are responsible for the most severe viral illness ever observed in tomatoes, others only exhibit aggressive symptoms when combined with other viruses (9). The potexvirus members can also be transmitted mechanically and through bumblebees (31). PepMV was initially isolated from pepino plants in Peru and later discovered to infect tomato plants in the Netherlands in 1999 (31). Currently, PepMV has been documented as the cause of newly occurring tomato infections worldwide (31). Our observation revealed a positive correlation between the percentage of infection in the field and the number of viruses discovered in tomato fields (table 1). Therefore, the inability to identify all the viruses that were reported on tomatoes in previous studies (3) demonstrated that the testing kits could detect the viruses at low concentrations, which aligns with an earlier report indicating that the concentrations of viruses in plant tissues impact the successful detection of viruses using immunostrip test (21). Previously, PVX, TAV, CMV, TBSV, GALV, TMV, ToMV, TSWV, TYLCV and ToLCPMV were reported on tomato crops in Iraq (3). This study utilized rapid, specific, and user-friendly method to acquire comprehensive insight into viruses

belonging to tobamovirus and potexvirus groups infecting tomato plants in Iraq. We have successfully detected eight different viruses infecting tomatoes in the middle and south of Iraq. The presence of these viruses was detected in either single or combined infections. The extracted samples yielded a uniform solution with a green or light brown tint as a sign of a well-extraction process. Positive outcomes may be seen within five minutes. However, many samples required the maximum recommended time of 30 minutes; this indicates that the virus infections in tomato fields were in low titers. In this investigation, we endeavored to detect the viruses of interest using the Pathogen ImmunoStrip Test specific to detect ToBRFV, TMV, PMMoV, CymMV, ORSV, CGMMV, PepMV and PVX on tomato plants. Four major tomato areas (Basrah, Nasiriyah, Najaf, and Karbala) were discovered to have a high incidence of viruses infecting tomatoes, while Diyala is evaluated with a medium level of virus incidence on tomatoes, and two governorates (Baghdad and Babylon) showed a low level of virus incidence on tomato plants (Figure 3). The trend line showed that the virus incidence is going high as we head south of the country towards the Arabian Gulf (Figure 3). Different tomato cultivation farms in Basrah were found to be infected with all eight viruses under investigation including the six distinct and newly reported viruses: CGMMV, CymMV, ORSV, PepMV, PMMoV, and ToBRFV. A single member of the tobamovirus group (ORSV) was discovered only in Basrah infecting six tomato cultivars. Prior reports have documented the infections of TMV, ToMV, PMMoV and ToBRFV in Iran and Turkey (27, 29), which raises the concern that the new emerging viruses confirmed in this study may have crossed the borders from the neighboring countries through agricultural materials trade. Only three mechanically transmitted viruses (TMV, ToMV and PVX) were previously documented in Iraq (3). The concern of having plant virus diseases spread in Iraq coming from other countries was raised previously when other viruses showed genetic relationships with isolates from these countries (2).

Incidence and distribution of tomato viruses

The viruses ToBRFV and CymMV were detected most frequently, being found in all seven governorates surveyed. Previous investigations have also documented its frequent presence in tomatoes in Turkey and Iran (13, 29). In this investigation, the newly detected viruses were found in all seven Iraqi governorates regardless of the tomato cultivar, suggesting that the distribution of the viruses is not limited by tomato cultivar. This finding is comparable with prior studies conducted in Iran (27). Furthermore, while comparing the infection distribution of the investigated viruses, it was found that PepMV and PMMoV were detected in 6 out of 7 surveyed governorates (table 1). The impact of this wide distribution of the four viruses was obvious. CGMMV and TMV shared the third most detected viruses using Immunostrips in this study. The two viruses were found in 5 out of 7 governorates, which aligns with a previous study that observed TMV on tomatoes cultivated in different governorates in Iraq (3). We identified a low incidence of two other viruses (ORSV and PVX). A noteworthy finding is the detection of ORSV, CGMMV, CymMV, PepMV, PMMoV and ToBRFV, potexviruses and tobamoviruses, infecting tomatoes in Iraq were investigated for the first time in this study. Previously, ToBRFV, PMMoV were found in Turkey and Iran (13, 29). However, in this study, ORSV was only found in Basrah, near the port. Increasing the possibility of having this virus entering Iraq through the trade of agricultural materials. A sequence of the infected tomato, particularly because it was detected in Iraq from tomatoes that showed no symptoms, would be highly encouraged. Many of the tomatoes sampled showed no symptoms, including those that were identified positive to several viruses. This observation can be attributed to the influence of genotypes or cultivars, such as Ostora, which are infected by eight viruses showing no or mild symptoms. Ostora is a tomato cultivar known as virus-resistant, and it typically shows no symptoms when infected with viruses. The data we collected from farmers regarding tomato production in the last three years showed a decrease in tomato yield reaching 50-70% in the southern governorates

of Naseriyah and Basrah (Figure 4). This puts more stress on Iraqi vegetable farmers who are already facing difficulties in the market (28). The long-term damage cannot be assessed at present, as a lot of information regarding these viruses in Iraq remains unknown. Agriculture in general faces a serious challenge because of the increasing damage by biotic stresses (7, 11, 15), and the attempt of finding ways to suppress this damage is continuing (32, 33). Tomato viruses pose a significant risk to the productivity and quality of tomatoes. Given that the plant is reproduced locally by seeds and through vegetative means, a crucial method for managing tomato viruses is the certification of tomato propagation material, as mandated by legislation. However, this is not true for the majority of the viruses belonging to the tobamovirus and potexvirus groups, which are the focus of our research. The lack of symptoms observed in the plants included in our experiment provides evidence of the minimal harm posed by these viruses to tomato production. Consequently, it is unlikely that these viruses will be included in certification schemes for the propagation of tomato cultivars. However, caution should be exercised when transferring agricultural materials, as it is the primary method for spreading tomato viruses.

Figure 1. Chlorotic and yellow blotches (A), followed by brown spots, and rugose wrinkles (B) and lines appearing on tomato fruits (C) were observed in tomato fields located in Diyala and Basrah. These plants were detected positive to ToBRFV

Figure 2. Common mosaic and mottling symptoms associated with the infection of TMV (left) and CymMV (right) were observed on tomato plants in different tomato cultivation areas in Iraq. The virus infection was confirmed serologically using immunostrip tests

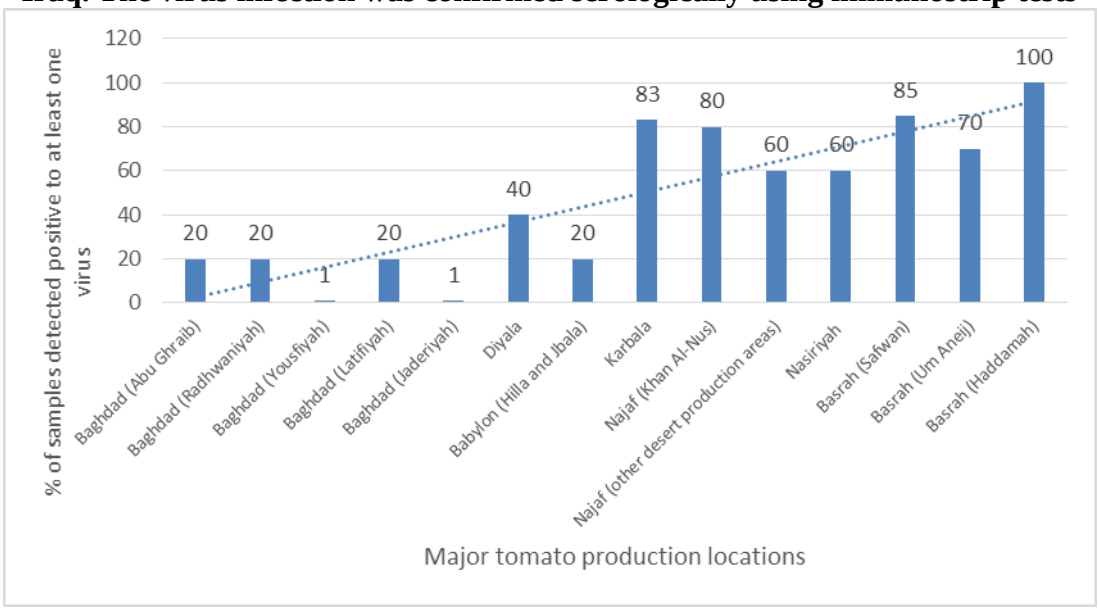

Figure 3. Percentage of samples detected positive to at least one virus of the eight tomato viruses under investigation in the major tomato production areas in Iraq. The trend line shows that the virus infection is higher as we head south of Iraq

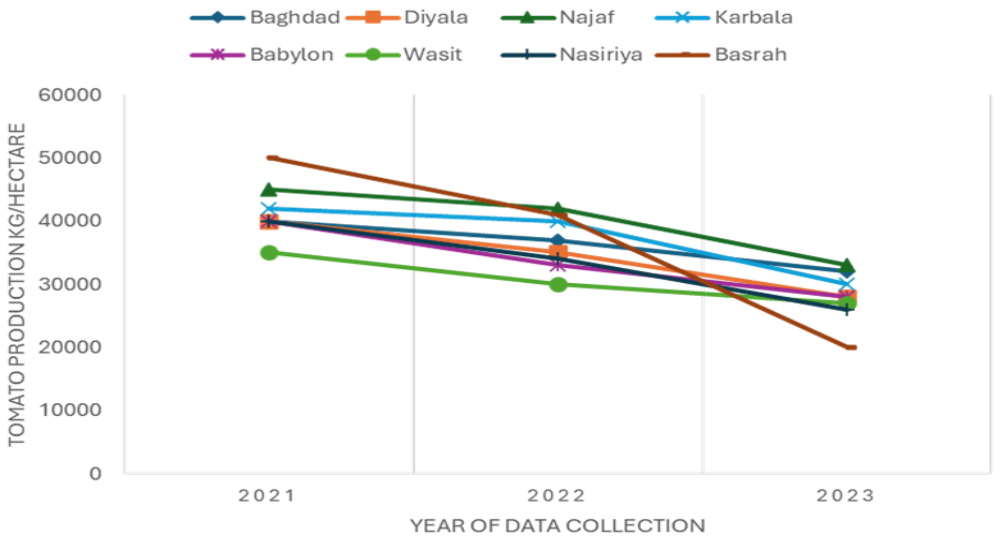

Figure 4. Tomato production (yield KG/ha) over three years course in seven main tomato production governorates in Iraq, based on data collected from tomato local growers

Table 1. Level of infections with the eight tobamo- and potexviruses in tomato in the surveyed locations in Iraq. The percentage of infection was also shown for each tomato cultivar.

Region	Cultivation type	Tomato cultivar	Positive for							(%) infection
			CGMMV	CymMV	ORSV	PepMV	PMMoV	PVX	TMV	ToBRFV	
Baghdad	Open field & Tunnels	Latevia	X	X		X		X		X	20%
		Madina				X				X	20%
		Raoa'a & unknown					X		X	X	20%
			X	X				X		X	1%
Diyala	Open field	Unknown	X	X						X	40%
Babylon	Tunnels	Madina				X	X			X	20%
Karbala	Open field	Mercury	X	X		X	X		X	X	100%
		Raoa'a	X	X		X	X		X	X	70%
		Bashira	X							X	80%
Najaf	Open field	Raoa'a		X			X				60%
		Basheer		X					X		80%
		Bushra		X							90%
		Coral		X			X		X		90%
		Jandar		X			X		X		80%
		Oscar		X		X			X	X	40%
Nasiriyah	Tunnels	Gxara	X	X		X	X	X	X	X	60%
		Mercury	X	X		X	X	X	X	X	60%
Basrah	Tunnels	Ibrahimi	X	X	X	X	X		X	X	60%
		Aseel		X	X	X	X		X		60%
		Ostora	X	X	X	X	X	X	X	X	100%
		Sultan		X	X	X	X		X		60%
		Yasmeen	X	X	X	X	X			X	100%
		Salima	X	X	X	X	X			X	100%

CONCLUSIONS

This study utilized rapid, specific, and user-friendly method to acquire comprehensive insight into viruses belonging to tobamovirus and potexvirus groups infecting tomato plants in Iraq. We have successfully detected eight different viruses infecting tomato plants in the main cultivation areas in the middle and south of Iraq. The presence of these viruses was detected in either single or combined infections. Six of the eight viruses (CGMMV, CymMV, ORSV, PepMV, PMMoV, and ToBRFV) are reported for the first time infecting tomatoes in Iraq.

Conflict of interest statement

The authors declare no conflict of interest

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