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DETERMINATION OF THE FIELD SPREAD OF *TOMATO SPOTTED WILT ORTHOTOSPOVIRUS* ON THE SOLANACEAE CROPS AND THE ASSOCIATED WEEDS IN NINEVEH PROVINCE

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Abstract

A survey has been conducted on two fields that are well-known for planting Solanaceae crops located in Nineveh province during the period extended from November to April, 2019. The aim behind the survey is to investigate the spread of the *Tomato Spotted Wilt Virus* (TSWV) on some crops of the Solanaceae including (tomato, eggplant pepper), in addition to some wild weeds accompanying these crops, which are considered a storage of the virus during the absence of its main families. To that end, about 348 samples showing virus-like symptoms have been collected covering plants of tomato, eggplant pepper, and the accompanying weeds as well. In this regard, the DAS-ELISA method has been used for diagnosing the virus by means of testing the presence of TSWV infection in the samples. The results of the ELISA test has shown that the presence of the virus is natural in the tested samples, and that the percentage of the presence of the virus in the field samples of the plants of Solanaceae, as a whole, has reached 27.2%. In this regard, it has been shown that the percentage of infection is different in accord with the kind of the plant. Thus, the highest infection percentage has been found in the eggplant corps, 40.2% and that in tomato and pepper corps has been 23.3% and 18.2%, respectively; whereas, the percentage of the presence of the virus in the whole 50 accompanying weed samples has recorded 63.03%. This research is the first recording of TSWV on crops of eggplant and pepper in Nineveh province and also it is the first recoding done, in Iraq, on secondary families with similar weeds.

Key words: TSWV, Survey, Solanaceae, Weeds.

Introduction

Solanaceae is an important place among cultivated species and others, such as potato, pepper and tomato, paly a determining role in the human diet and the economy of the countries (Souiri *et al*., 2020). Others are widely grown as ornametal plants or for industrial and pharmaceutical purposes (Knapp *et al*., 2004). Crops belonging to this family, such as tomato, eggplant and pepper, occupy a great nutritional important in Iraq, so the cultivated area rached (11.087.25 hictars) with these crops in Nineveh province (A.S.C, 2019). The problems of pests and plant pathogens, including viruses, have a serious economic determinant of crops grown as they reduce the quantity and quality of the world‘s crops (Gergerich & Dolja, 2006). *Tomato spotted wilt virus* (TSWV) is a member of the genus *Orthotospovirus* in the family *Tospoviridae* (Maes *et al*., 2018) is one of the most widely spread plant viruses and causal agent of economically important yield losses in many crops (Mumford *et al*., 1996). It has a broad host range, with more than 1090 plant species and also most of the plants listed as hosts of TSWV belong to family Asteraceae (247 species), Solanaceae (172 species) and Fabaceae (60 species) (parrella, *et al.,* 2003; Peters, 2004). In general, the two main cocomitant factors that make TSWV a complex agricultural pathogenic system are the highly polyphagous nature of its vectors and the relative lack of host specificity of the virus. Consequently, TSWV has a very large host range and its control remains problematic. TSWV is transmitted exclusively by least nine species of thrips, which must acquire the virus in larval stage, particularly first instar larvae, in order to be a vector in a propagative -circulative mannar, the virus is also transmitted mechanically by sap inoculation (Wijkamp *et al*., 1993; Mumford *et al*., 1996). TSWV has been recorded in many Arab countries (Jordan-Anfoka *et al*., 2006; Iraq-Chiro, 2010; Kuwait - AL-Ali *et al*., 2013;

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Syria- Halaby, 2014; occupied Palestine, Saudi Arabia, Algeria, Tunisia, Egypt, Libya, Morocco and Sudan - EPPO/CABI, 1997). Also the virus recorded in Turkey (Ozdemir *et al.,* 2009) and Iran (Hajiabadi *et al.,* 2012). The present study aimed at investigating the TSWV on some crops of the Solanaceae family in Nineveh province , and determine the presence of virus in some likely weed species that may act as its reservoir.

Materials and methods

Survey and samples collection: The survey was conducted on two farmas located in three main production areas in Nineveh agricultural districts. Each farms was visited two times during the production season, for the period from November to May 2019. During these tours, a number of samples have been collected selectively is (348), distributed among tomato, pepper and eggplant, these samples showing virus -like symptoms such as crinkling and deformation, mosaic as well as typical TSWV symptoms such as severe distorted leaves, chlorotic, vein necrosis, brown spotting and wilting, Fig. 1 and 50 samples inocluding the weeds accopanying theses plants, which most likely did not show any symptoms. The samples have been preserved in polyethlene bags after recording information on them, and were brought to the laboratory and placed in freezer at -18^{0}C , for the virus identification.

Virus identification by serological assays

Samples of symptomatic and non-symptomatic, plants were tested for virus infection by DAS-ELISA (supplied by Bioreba, Switzerland) The leaves (1g) were

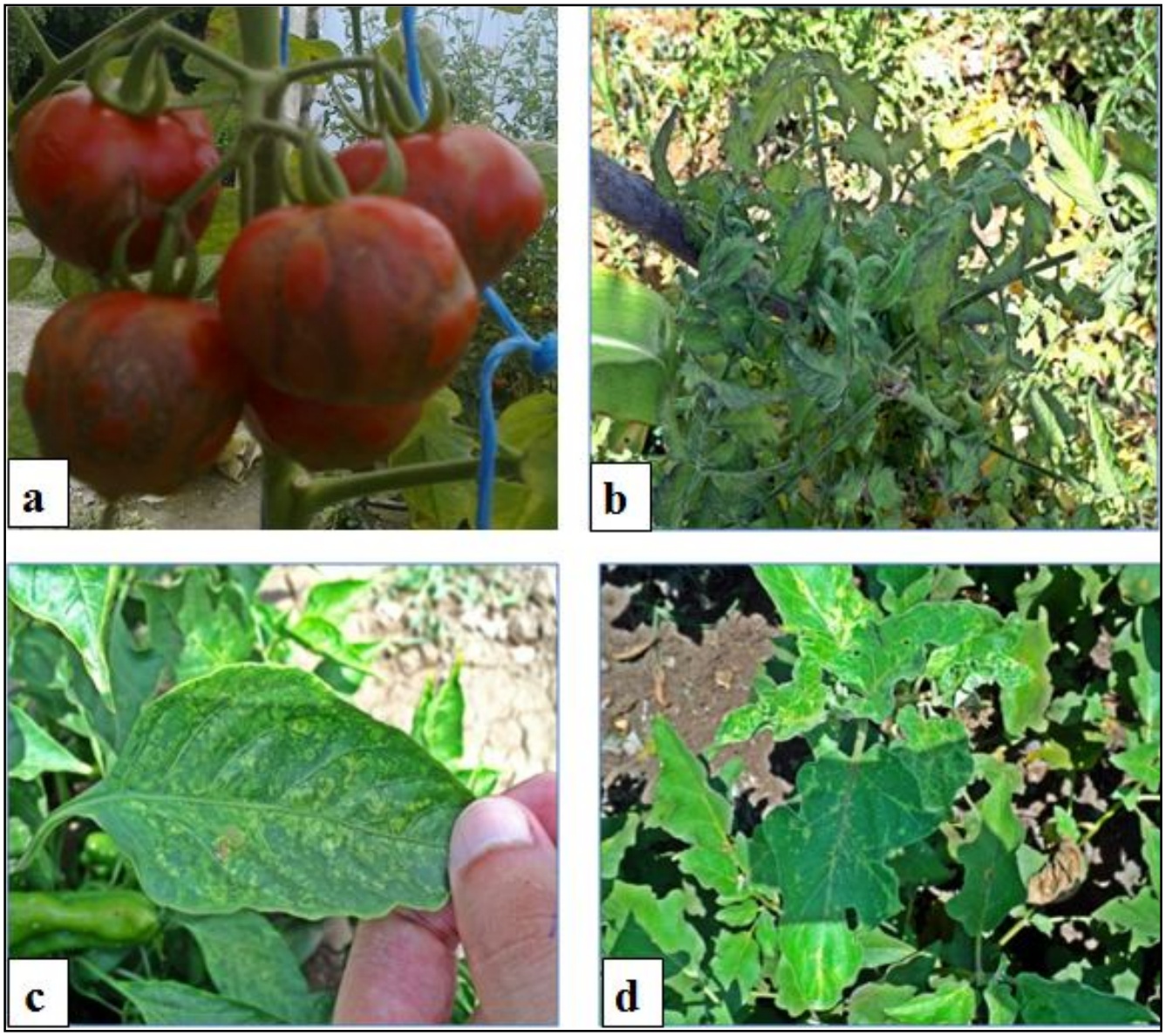

Fig. 1: Natural symptoms of TSWV on some solanaceae plants (a- tomato fruits, b- tomato, c-pepper, d- eggplant).

homogenized with 1 ml of the general extraction buffer in mortar and pestle. The homogenate was passed through 2 layers of muslin cloth and filtrate was used for DAS-ELISA reactions as following (according to the company instructions) :

1- Preparing humid box: a humid box was prepared by lining an airtight container with a wet paper towel. Test wells (micro ELISA plate) were kept in a humid box during a required incubation will help prevent sample from evaporating and dryness (Kasmi & Ali, 2012).

2- Preparing capture antibody: One hundred µl of anti TSWV IgG was added to 10 ml carbonate coating buffer for preparing capture antibody solution. The prepared was mixed and used immediately. One hundred µl of capture antibody solution were added into each well of ELISA microtiter plate.

3- The plate was maintained in a humid box overnight at 4ºC.

4- The wells were rinsed several times with 1X PBST.

5- One hundred µl of plant extract, were added in to each well and maintained in the humid box at 37ºC for 2 hours in the incubater.

6- The wells were rinsed several times with 1X PBST.

7- Adding IgG -conjugate enzyme (IgG- conjugated with alkaline phosphatase must be diluted with enzyme conjugate buffer before use). One hundred µl of prepared IgG conjugate enzyme were added into each well and the plate was inocubated for 3 hours at 37ºC in humid box.

8- The wells were rinsed several times with 1X PBST.

9- Preparing and adding polyvinyl pyrolidone (PNP) solution: each PNP tablet was dissolved to make 5ml of NPN solution, at a concentration of 1mg/ml, about 15 min. before the end of the above incubation step (7). Then, without touching the tablets, were added to the buffer and without expose the PNP solution to strong light because of light or contamination could cause background color in negative weels. Then, one hundred µl of PNP substrate were added into each well and the plate was incubated for 60 min. at 37ºC.

10- Evalating results test: the wells were examined by a microplat reader (UV-9200 Spectrophotometer) at 405 nm. after 60-120 min. the development of yellow color indicates positive reaction, whereas wells in which there is not significant yellow color developed indicate negative result. And samples, were considered TSWV infected if their absorbans values, were greater than at least two time than of healthy control. Virus free plants were served as negative control.

Results and discussion

The results of DAS- ELISAserological test, for the collected samples during filed survey confirm the presence of the TSWV naturally with the samples . The combined infection rate reached (27.2%) on the solanaceae crops including (tomato, eggplant, pepper) and the infection rate varied according to the type of plant, as the highest infection rate was reached on the eggplant 40.2%, followed by 23.2 and 18.2 on tomato and pepper crops respectively. Futhermore, in this study a total of fifty samples belonging to the most weed plants included (*Amaranthus retroflexus*/Amaranthaceae; *Convolvulus arvensis*/Convolvulaceae; *Chenopodium album*/ Chenopodiaceae; *Portulaca oleraceae*/Portulacaceae; *Inula viscosa and Xanthium strumarium*/Asteraceae; *Alopecurus moysuroids*/Poaceae) Fig. 2 were tested by ELISA for the presence of TSWV, 6 species with the rate of 60.03% were found to be infected by TSWV table 1. Perhaps the reason for the high rate of infection with the virus in the weed samples is due to the spread of the thrips insects that transmit the virus to these weeds; infected weeds to be a major storehouse for a large number of type of dangerous viruses that can be transferred from them to economic plants cultivated near them, especially and transport is through vectors and the most dangerous and most active of these carrieres are insects. Thus, through this transfer, viruses achieve their goal of survival and continuation of the existing ecosystem through the process of tandem interchangeability from a wild weeds to an economic plant, then back to the weeds. Thus, the reciprocal cycle of the virus and the insect vector is the main player in it. And what confirms the danger of these weeds , as no symptoms appear on them, thus representing a source of continuous damage in the field without any sign of infection. The results of Ozdemir *et al*., (2009), indicated the presence of TSWV on 43 leaf samples tested by ELISA out of 71 tomato leaf samples and all fruit samples were found to be infected by the virus , the infection rate was 100%. On the other hand in a survey study in the open and protected cultivars of Syrian , Halabi *et al*., (2013) has found that the weeds belonging to the many plant families have been carrying the TSWV. While Ozdemir *et al*., (2009) scored in a study conducted in Denizli province, Turkey to investigate the TSWV on weeds, a total of 41 weeds samples, belonging to ten plant families, were found for infected of the virus. Wild weeds play multidimensional role in the epidemiological spread of the virus (Duffus, 1971; Groves *et al*., 2002). This research is the first record of the TSWV

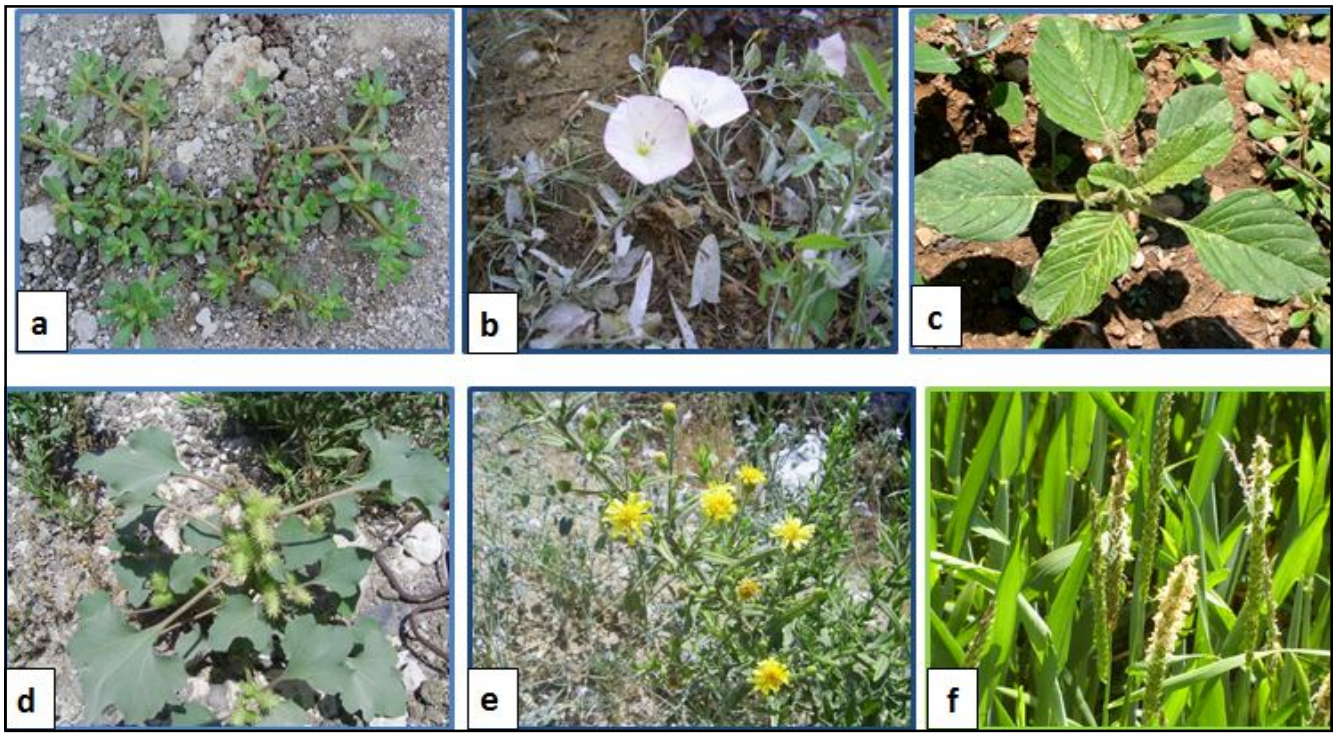

Fig. 2: Important weeds found in tomato fields and infected *Tomato spotted wilt virus* (a- *Portulaca oleraceae*, b- *Convolvulus arvensis*, c- *Amaranthus retroflexus*, d- *Xanthium strumarium*, e- *Inula viscosa*, f-*Alopecurus moysuroids*

Table 1: Results of DAS-ELISA test, for the diesease incident of *Tomato spotted wilt virus* on some solanaceae crops and associted weeds for different areas in Nineveh Province.

Crops	Family	Growth habit	Total tested plants		Number infected plants		Infected plants (%)		*Mean of ELISA values
			With symp-toms	Without symp-toms	With symp-toms	Without symp-toms	With symp-toms	Without symp-toms	
Solanum lycopersicum L.	Solanaceae	Annual	103	0	24	0	23.3	0	0.155
Capsium annum L		Annual	93	0	17	0	18.2	0	0.124
Solanum melongena L.		Annual	102	0	41	0	40.2	0	0.379
Total			298	0	82	0	27.2	0	Cont. 0.970
Amaranthus retroflexus L.	Amaranthaceae	Annual	0	6	0	2	0	33.3	0.082
Convolvulus arvensis L	Convolvulaceae	Perennial	0	7	0	5	0	71.4	0.115
Chenopodium album L.	Chenopodiaceae	Annual	0	6	0	0	0	0.0	0.001
Portulaca oleraceae L.	Portulacaceae	Annual	0	9	0	7	0	77.7	0.162
Inula viscosa L	Asteraceae	Perennial	0	8	0	7	0	87.5	0.211
Xanthium strumarium L.		Annual	0	8	0	6	0	75.0	0.113
Alopecurus moysuroids Huds.	Poaceae	Annual	0	6	0	3	0	33.3	0.087
Total			0	50	0	30	0	63.03	Cont. 0.970

*Absorbent at 405 nm, each value represent the mean of three reading.

on eggplant and pepper in Nineveh Province, also it is the first registration of several new herbaceous plants in the Iraqi envornment belonging to five families inocluding (*Amaranthus retroflexus*; *Convolvulus arvensis*; *Portulaca oleraceae*; *Inula viscosa; Xanthium strumarium and Alopecurus moysuroids*), repersenting reservoir and secondary host for TSWV.

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