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SEROLOGICAL AND BIOLOGICAL DETECTION OF *TOMATO MOSAIC VIRUS* IN SOIL AND SEEDS OF TOMATO PLANTS IN DUHOK PROVINCE*

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ABSTRACT

***Tomato mosaic virus* (ToMV) is one of the most important viruses on tomato; contaminated soil may be the main source and reservoir of transmission. Diseased plants were collected from Kamona and Xanike villages, Duhok province. DAS-ELISA technique its detection in the soil and contaminated seeds, and clarify the virus transmission from contaminated seeds to seedlings. Using indicator plants (Biological detection) was to identify virus from contaminated soil. The result showed that six samples out of 14 soil samples gave a positive reaction with 42.8% is transmission ratio from soil. Symptoms on indicator plants such as: mosaic, malformation, vein clearing on *C. qinoa*; the stunted plants with localized necrotic lesions and malformation on pepper plants. DAS-ELISA from tomato seeds displayed in 11 samples out of 18 samples revealed strong reactions and seeds transmission proportion were 61%. Seedlings were germinated from 60 infected tomato seeds under plastic house condition but only 4 transplants exhibited ToMV symptoms of mosaic, and the percentage of transmission was 7.8%.**

***KEYWORDS**:* Tomato, Tomato mosaic virus, DAS-ELISA
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INTRODUCTION

Tomato *(Solanum lycopersicum*), Solanaceae. Is the most widely vegetable in the world, and the common components of the Mediterranean food, (Dorais *et al*., 2001; Chookhampaeng *et al*., 2008). It considered to be the second greatest important product after potato in the world, and an excellent source of healthy promoting agents due to contain of balanced mixture of vitamin C and E, antioxidants, lycopene, lutein, B-carotene, flavonoids and minerals in its contents, (Dorais *et al*., 2008).

The viral diseases have been ranked as one of the important diseases of tomato, (Petrov, 2014). About 130 viruses are known to infect tomato worldwide, (Hanssen *et al*., 2010) and cause between 20-90% losses in production, (Hameed, 1995).*Tomato mosaic virus* (ToMV) is the most important *Tobamoviruses* infecting tomato (Hanssen, *et al*., 2010; Li, *et al*., 2013). ToMV belongs to *Tobamovirus* genus (Francki *et al*., 1985) and belong to the family *Virgaviridae* (King, 2011). It is stable RNA virus wide-spread in distribution and infects many plant species (Hollings and Huttinga, 1976). Four strains of ToMV were recognized on tomato (Tm-0, Tm-1, Tm-2, Tm-2^2) based on resistance (R) genes (Pelham, 1966; Hall, 1980).

Symptoms of infected tomato crop including appearance of curling, mosaic, leaf distortion, uneven ripening and internal browning of fruit. In susceptible cultivar ToMV provokes serious disease in tomato plant drastically reducing yield (Najeeb Ullah, *et al*., 2017). Average incidence of disease caused by this virus ranged between 25.49- 29.79% in tomato seeds and leaves (Khan, 1997). While Arinaitwe (2013) found that the incidences of ToMV were more than 60% in field samples with high level of mixed virus infection.

Under adverse environmental condition ToMV is quite stable and can persist in moist soil for a month or in dry soil for two years or in fallow soil at 120cm in root debris for 22 months, (Yadav and Yadav, 2017).

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Contaminated soils play roles as reservoirs and source of ToMV transmission (Yang *et al*., (2012). *Tobamoviruses* stay in soil with plant debris. Therefore, the soil serves a primary source of inoculum of later crops (Broadbent, 1965; Nagai, 1981; Lanter *et al*., 1983; Pares and Gunn, 1989). The viral particles are identified to be absorbed to organic plant debris and clay particles, (Kegler *et al*., 1995). ToMV can be transport through soil and water from diseased to healthy plant; then can enter the plant via roots and cause infection, (Schwarz *et al*., 2010).

ToMV is seed-borne and seed transmission (Broadbent, 1976; Gooding and Suggs, 1976; Chitra *et al*., 1999).Virus contamination the seed coat of tomato and usually transfer externally (Pradhanang, 2005). The virus is present in external and sometimes endosperm of tomato seed, but was not showed within embryo (Broadbent, 1976).

ToMV transmission by tomato seed has been study widely (de Assis Filho and Sherwood, 2000), the rate of seed transmission of ToMV reach up to 94% while less than 1% seed transmission rate is enough to serve as inoculum for epidemic due to mechanical transmission (Broadbent L.1965; Brunt, *et al*., 1997).

The current work aimed to identify ToMV on symptomatic tomato plants using DAS-ELISA (double antibody sandwich-enzyme linked immunosorbent assay) and determine its transmission by soil and tomato seeds at first time in Duhok province.

MATERIALS AND METHODS

Field survey to detect ToMV from soil sample and tomato fruit

Soil samples were collected in October 2018 from the selected tomato fields surveying place in Semel, Duhok Province in the area of Khanke and Kamona villages. Each sample weighed 2-3 kg, was taken from the rhizosphere of infected tomato plants at 5-30cm soil depth. Samples were bagged, labeled properly and transferred to the laboratory of Plant Pathology, collage of Agriculture Engineering Sciences, University of Duhok.

During the field visits, 18 symptomatic tomato plants were selected, after confirming that the virus has detected by DAS-ELISA test, each plant were labeled until harvesting. The fruits were also collected at full ripening. Soil samples, tomato plants and fruits were used for detection ToMV using DAS-ELISA.

Examining the presence of the ToMV in soil samples

DAS-ELISA method was used to detect ToMV in soil samples, according to Clark and Adams (1977) and instructions of antiserum manufacture ("Agdia", France)

Capture antibody (IgG) solution was prepared and diluted with 10 ml carbonate coating buffer, mixed with 50 µl of capture antibody, and pipette 100 µl into each well. The plate incubates for 4 hrs. at room temperature before washing by PBS-Tween two times. Mixed soil sub-sample 50gm was taken and washed with 100ml of phosphate buffer plus drops of Tween. The samples are shaken on the rotary shaker (200 rpm) for 45min; the soil extract was filtered with a double layered of muslin cloth in a clean beaker and 5ml of soil extraction was mixed with 1ml of general extract buffer (GEB). Plate incubates for 2 hour at room temperature, then washed 7 times as mentioned previously. Prepare enzyme conjugate 10 minutes before use: you should be added 10ml ECI (enzyme conjugate) buffer with 50 µl of enzyme conjugate. Incubate plate for 2 hour at room temperature. Plate washed 8 times with PBST. Prepared PNP solution: each PNP tablet made 5 ml of PNP solution, and dispensed 100 µl of PNP substrate into each wells. Incubated the plate for 60 minutes.

Examining the presence of the virus in soil samples biologically using bait plants

The ToMV infested soil samples were transported into pots 13 cm in diameter. Healthy seeds of each pepper (*Capsicum annum*) and goosefoots plants (*Chenopodium quinoa*) were planted at a rate of 5seeds/pot (Buttner and Nienhuase, 1980), to determine whether the with virus presence. The experiment was conducted under plastic house in collage of Agriculture during 2018. Plants were observed visually for checking the appearance of viral symptoms.

Examining the presence of the virus in tomato GC cultivar seeds by DAS-ELISA

ELISA was reliable technique and very sensitive for detection of virus, nowadays suitable for testing seeds (Morrison, 1999). DAS-ELISA method was used to detect ToMV in tomato seed, according to Clark and Adams (1977) and instructions of antiserum manufacture ("Agdia", France).

Full ripening tomato fruit were crushed to obtain seeds. Five infected tomato seeds from each sample were grounded by pestle and mortar blinded with General Extract Buffer (GEB) at pH 7.4, at ratio of 5:10 (W/V) then passed through double layered muslin cloth to remove the seed derbies and then diagnosed by DAS-ELISA.

Examining the percentage of natural transmission of the virus by seeds

Fully ripped tomato fruits (GC hybrid) were crushed to obtain seeds, 60 seeds of infected tomato were planted in sterilized container wooden box (50x20 cm) containing of autoclaved soil and peatmoss (3:1, w/w) in lines 10 cm interval with 5cm between seeds .This experiment was conducted in the green house at Collage of Agriculture Engineering Sciences, University of Duhok. The plants were maintained for up 2 months to observe the appearance of systematic symptoms (Sevik, and Tohumcu, 2011).

RESULTS AND DISCUSSIONS

Detection of virus in soil samples by DAS-ELISA:

The results showed that six samples out of 14 soil samples gave a positive reactions with 42.8% soil transmission rate. Due to reasons for example soil infestation in previous season was more so soil infestation with virus will be more.

The positive DAS-ELISA characterized by the yellow color of substrate solution as shown in (Fig. 1). In this aspect Yang *et al*., (2012) and Fillhart, *et al*., (1998) were applied DAS-ELISA for detection ToMV in the soil with distinct rate of 5%.

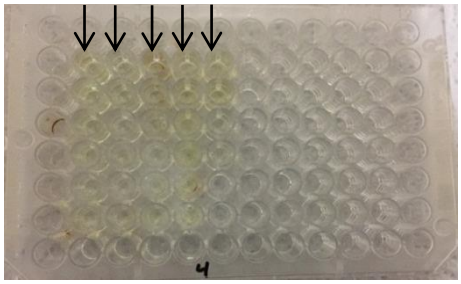

Fig. (1): DAS-ELISA test, Appearance of yellow color in the 2nd to 4th columns which indicates the presences of ToMV in soil samples.

Biological detection of ToMV using indicator plants

The virulent ToMV particles in tested soils resulted in varied symptomatic plants of *Chenopodium quinoa* and pepper (*Capsicum annum*) after four weeks of inoculation (Fig.2).

Symptoms included mosaic, malformation, vein clearing on pepper plants; the stunted plants with necrotic lesions were most common. These symptoms were also described by Madhusdhhan *et al*., (2005). Kumar *et al*., (2011) observed symptoms of leaf curling, mosaic and stunted growth on pepper. In contrast the necrotic lesions were developed on *C. qinoa* (Fillhart *et al*., (1998).

Figure (2): Biological detection of ToMV from soil, appearance the symptoms of ToMV on *Chenopodium quinoa* (A) and pepper plant (B).

Detection of ToMV in tomato seeds by DAS-ELISA

The ToMV survived in seeds identified by using DAS-ELISA. Eleven samples out of 18 samples revealed strong reactions (Fig.3). The percentage of the literatures demonstrated the highest occurrence ToMV of infected tomato seeds reached 61%. Almeida *et al*., (2018) and Van Winckle and Gcypens (1965) were reviewed the frequency of viral transmission by tomato seeds reached to 98% and 94% respectively, Hadas *et al*., (2004) reported that ToMV contaminated 78.8% tomato seeds. Reasons may be the

differences in infection percentages is attributed to the different cultivars of tomato studied.

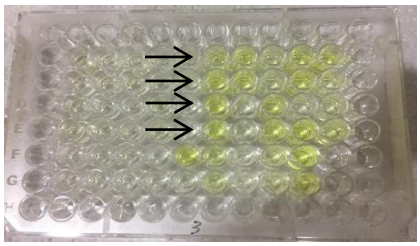

Fig (3): DAS-ELISA test. Appearance of yellow color in the 7th to 11en columns, indicated the presences of ToMV in mature tomato seeds.

Estimation of natural transmission percentage of the virus by seeds

Seedlings were germinated from 60 infected tomato seeds under plastic house conditions but only 4 exhibited ToMV symptoms of them (mosaic) as shown in (Fig.4), the virus succeeded to pass from seed coat to penetrate seedlings tissues causing systemic infection and the percentage of transmission was 7.8%. The similar results were found by (Sevİk, and Kose-tohumcu, (2011) with the rate transmission of 23.5%.

Fig. (4): Transmission of ToMV from seeds to seedling test, as shown by symptoms of ToMV .

CONCLUSION

We concluded that the ELISA test showed high efficiency in detection *Tomato mosaic virus* (ToMV) in soil samples, and also in tomato seeds obtained from infected fruits, the percentage of its presence in soil samples were 42.8%, and in seeds 61%.

The indicator plants of pepper (*Capsicum annuum*) and goosefoots (*Chenopodium quinoa*) were successfully used for the purpose of catching of ToMV from infested soil , because they infected systemically when planted in such soil, The biological test had been validated in the detection of ToMV in soil. The results of presence ToMV in the contaminated tomato seeds revealed the restricted ability of a pathogen to pass from seed coat to tomato seedlings and not exceed than 7.8% when planted contaminated seeds in sterile soil. Thus, the high rate of infested seeds with ToMV.

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ئاشکەراکرنا سێرولوجی و بایولوجی یا جنجربوونا باجان سورکێ د ئاخێ دا و د تووڤی دا سەر رووەکێ باجانێ ل پارێزگەها دهوکێ

پوختە

نەخوشییێن ڤایروسی دهێتە هژمارتن ژ گرنگترین ئاستەنگێن چاندنا ڤی بەرهەمی ل جیهانێ , ڤایروسێ موزاییك یێ تەماتێ دهێتە لسەری هەمی ڤایروساتا ژبەر گرنگیا وێ. ئاخا پیسبووی ب ڤایروسی ئێکە ژ گرنگترین رێکێن هەلگرتن و ڤەگوهاستنا ڤایروسێ. من نموونێن نساخ کوم کرن ژ بیسانێن گوندێ کەمونا و خانکێ . بکارئینانا تەکنیکی یا الیزا بو ئاشکەراکرنا ڤی ڤایروسی د نموونێن ئاخێ و تووڤیدا, چونکە ئەڤ ڤایروسە دهێتە ڤەگوهاستن برێکا تووڤی بو شتلێن ژێ شین دبن. و ئاشکەراکرنا ڤایروسی دنموونان دا هاتە کرن بکارئینانا رووکێن ئاشکەراکری یت خەفك کری (ئاشکەراکرنا بایولوجی) .د ئەزموونکرنا الیزا دا کارلێکا موجەب هاتە کرن د شەش نموونان دا ژ 14 نموونێن ئاخێ ێن لابوری, و برێژا 42.8 % . بەلێ با دگەل نموننێن تووڤی بتنێ کارلێك هاتە ئەنجامدان دگەل 11 نموونا ژ 18 نموونێن توڤی ێن لابوری , برێژا ڤەگوستنا بتووڤی گەهشتە 61% . ب چاندنا 60 تووڤێن تەماتێ یت پیسبووی ب ڤایروسی د سندوقەکا داری دا, 51 ژوان شین بین بوونە شتل, نیشانێن ڤایروسی بتنێ لسەر 4 شتلا دەرکەفتن, ژبەرهندێ رێژا ڤەگوهاستنا تووڤی بو شتلا 7.8% دەرکەفت. هەروەسا نیشانێن ڤایروسی دیاربی لسەر رووەکێن ئاشکەراکری ین خەفککری ئەوژی سمرکە و فلفل پشتی چار حەفتیا ژ چاندنێ, وەکی دیاربی نیشانێن موزاییك, تێکچوون, و ئاشکەراکرنا دەمارێن بەلگی لسەر یا ئێکێ, و زیپکێت مری و کورتاتی دەرکەت لسەر یا دووێ.

الكشف الحيوي والمصلي عن فايروس موزائيك الطماطة *Tomato mosaic virus* في التربة والبذور في محافظة دهوك

الخلاصة

تعد الأمراض الفايروسية من أهم محددات زراعة هذا المحصول عالميا، ويأتي فايروس موزائيك الطماطة *Tomato mosaic virus* على رأس هذه الفايروسات أهمية. يعود هذا الفايروس الى الجنس *Tobamovirus*، وقد تكون التربة الملوثة به هي أحدى أهم وسائل الاحتفاظ به ونشره. جمعت العينات المصابة من حقول قريتي "كمونة" و Xanike . واستعملت تقانة DAS-ELISA للكشف عن الفايروس في عينات التربة والبذور، وذلك لأن الفايروس ينقل من البذور الملوثة به الى البدرات الناتجة. وتم تشخيص الفايروس في

عينات أيضا باستعمال النباتات الكاشفة الصائدة (الكشف الحيوي). أعطى اختبار DAS-ELISA تفاعل موجب مع ستة عينات من أصل 14 عينة مختبرة من عينات التربة، أي بنسبة 42,8%. أما مع عينات البذور فقد أعطى هذا الاختبار تفاعل موجب مع 11 عينة من أصل 18 عينة مختبرة ، أي بنسبة نقل بالبذور وصلت الى 61%. وزرعت 60 بذرة طماطة في صندوق خشبي ، نبت منها 51 بادرة ، وظهر على أربعة منها فقط أعراض الفايروس ، وبذلك فان نسبة النقل من البذور الى البادرات هي 7,8%. وظهرت على النباتات الكاشفة الصائدة وهي الرغيلة *Chenopodium quinoa* والفلفل *Capsicum annum* أعراض الفايروس وذلك بعد أربعة أسابيع من الزراعة، حيث ظهرت أعراض الموزائيك والتشوه ووضوح العروق على الأول، فيما ظهرت أعراض البقع الميتة والتقزم على الثاني.