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FIRST RECORD OF *DAHLIA MOSAIC VIRUS* IN IRAQ ON DAHLIA (*Dahlia pinnata*).

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ABSTRACT

The presence of Dahlia *mosaic virus* (DMV) was confirmed for the first time on both leaves and tubers of *Dahlia pinnata* in Iraq. DMV was confirmed by amplification of opening reading frame 1 (ORF1) that is encoding for the movement protein, using the specific primer (DMV F1 and DMV R1). The expected size of about 900 bps was amplified from 30 samples. There was no evidence of infection with DMV-D10 and *dahlia common mosaic virus*. Mechanical and vector transmission studied as biological properties of the virus. *D. pinnata, Zinnia elegans, Ageratum conyzoides* cv. Blue Ball, *Amaranthus caudates, Helianthus annuus* and *Chrysanthemum indicum* were used as indicator plants. DMV was mechanically transmissible to all tested plants, at a rate of about 80%. The diseased dahlia was tested for the presence of viral infections with, TSV, TRV, CMV and TSWV using *Chenopodium quinoa* and *Nicotiana tabacum* as diagnostic species. Both species remained symptomless after several inoculations. DMV was transmitted by *Myzus persicae* and *Aphis fabae* at the rate of 60 and 55 %, respectively.

KEYWORD: *Dahlia pinnata*, *Dahlia mosaic virus*, PCR, Biological studies.
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INTRODUCTION

Dahlia (*Dahlia pinnata*) is an ornamental and flowering plant, with tuberous roots in *Dahlia* genus and a member of Asteraceae family. The common garden variety of dahlia is an important root crop and medicinal plant. Its roots valued for the nutritious inulin and the antibiotic compounds concentrated in the tubers skin (Whitley, 1985). Dahlia plants reproduce asexually by vegetative tuberous roots, therefore it is more susceptible to viral infections. *Dahlia mosaic virus* (DMV) is the distinct caulimovirus that is associated with dahlia mosaic diseases (Eid and Pappu, 2014). It is the most important and prevalent plant pathogenic virus that has economic impact on flower production of dahlia (Pappu *et al.*, 2005). The virus first reported in *D. pinnata* from Germany in 1928 (Brunt, 1971). It is a member of *caulimoviridae* family that consists of double stranded DNA (dsDNA) genome. DMV virions are 48-50 nm in diameter and the genome size is about 7 killobase pairs (Brunt *et al.*, 1996). There is very little known about disease incidence of the virus and geographically widespread in dahlia growing areas and the natural host range is limited to *Dahlia* spp. (Pappu *et al.*, 2005). Under experimental conditions, several families, including Amaranthaceae, chenopodiaceae, compositae are susceptible to infection by DMV. The susceptibility to DMV is found in *Verbesina encelioides*, *Ageratum conyzoides*, *Zinnia elegan*, *Amarantus caudatus* and *Chenopodium capitatum* (Brunt, 1971). One of the important methods that transmit the virus is vector transmission. All viruses of caulimoviridae are transmitted by aphids, mealybugs and leafhoppers (Blanc, 2008). Aphid is the common vector of DMV. The study was conducted to diagnose DMV for first time in Kurdistan-Iraq, using biological method and PCR technique.

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MATERIALS AND METHODS

Seeds of dahlia (*Dahlia pinnata* Waterlily cv.) seeds have been used throughout the investigation. The sterilized seeds were cultured in pots (20 cm in diameter) containing sterilized culture substrate (sandy soil and peat-moss 3:1). The pots maintained in greenhouse, under normal conditions, to prevent other viral contaminants transmitted by insects. The plants adequately irrigated and fertilized as needed to avoid any symptoms of water stress and nutrient deficiency.

Sampling and propagation of the virus isolate

In spring of 2016 and 2017, young leaf samples from symptomatic dahlia plants with mosaic, systemic vein clearing, necrotic spots, stunting and malformation were collected from various gardens and nurseries of Duhok governorate. The collected leaves were used as virus isolate. The developed plants from cultured seeds were inoculated mechanically with virus isolate that was prepared by using phosphate buffer at 7.5pH, containing 0.05 M KH_2Po_4. The newly formed symptomatic leaves were taken 45 days after inoculation and prepared for viral detection (Nerway *et al.*, 2012).

Molecular diagnosis of *Dahlia mosaic virus* using polymerase chain reaction (PCR)

The presence of DMV was detected in inoculated dahlia leaves and tubers using PCR. In this way, DNA extracted manually and then used for detection of three caulimoviruses associated with dahlia; *Dahlia mosaic virus* (DMV), an endogenous plant pararetroviral sequence DMV-D10, and *Dahlia common mosaic virus* (DCMV). PCR was performed using the specific primers pairs (DMV F1/R1, D10F1/R1 and DCMV F2/R2) as shown in Table 1 to detect the incidence of the three caulimoviruses DMV, DMV-D10 and DCMV, respectively. DNA of the viral infected plants extracted depending on that of Pappu *et al.* (2005) and Pahalawatta *et al.* (2008a). Final DNA pellet resuspended in 100 µL of water and stored at -20ºC. Each 20 µl PCR reaction contained 1 µl of the total Extracted DNA, 4 µl of red load mastermix (20 mM Tris, pH 8.4 and 500 mM KCl, 150 µM dNTP mix, 2 mM $MgCl_2$ and 0.1 µl Taq DNA polymerase), 1 µl each of forward and reverse primers, 13 µl sterile H_2O. PCR amplification done in a DNA thermal cycler programmed for 4 min at 94 °C for initial denaturation; 40 cycles each consisting of 94 °C for 30 seconds (s), and 30s extension per 1000 bp product at 72 °C; followed by a final extension for 7 min at 72 °C. Annealing temperature varied depending on the specific primer pairs (Pappu *et al.*, 2005 and Abdel-Salam *et al.*, 2010). Annealing temperature varied depending on the specific primer pairs. The PCR products analyzed by 1.0% agarose gel electrophoresis using 1x Tris-Boric-EDTA (TBE) buffer (Abdel-Salam *et al.*, 2010).

Table (1): Specific Primer Pairs to Detect Dahlia Viruses.

Primers	Sequence (5´-3´)	Expected Size (bp)
DMV F1	ATG AAT ATC TTA GAA AGG AA	939
DMV R1	CTT AAT CCT TAA GTT ATC AA	
DMV-D10 F1	ATGGATCGTAAAGAT T	900
DMV-D10 R1	CTG TTT TTC TGT GTT TCT ACT GG	
DCMV F2	ACA GGA GTT AAA AGC TGG	646
DCMV R2	TCG AGG ATA TTG TTG TTG	

Biological assays

Symptomatology. The indicator plants used for viral symptomatology were *Dahlia pinnata*, *Ageratum conyzoides* cv. Blue ball, *Zinnia elegans*, *Chenopodium quinoa* and *Amaranthus caudatus* (Brunt, 1971) in addition to *Nicotiana tabacum*, *Helianthus annuus* and *Chrysanthemum indicum*. All these plants were inoculated mechanically with the virus isolate. The seeds of these plants were cultured in pots containing sterilized soil and peatmoss (3:1, v:v) and maintained in greenhouse. The developed plants, when reached to an optimum length with four fully expanded leaves, were inoculated with virus inocula. The plants had been left under observation for a month to odvice any symptoms may appear on them.

Transmission by aphids. Two aphid species belong to Aphididae were used in this trail, namely green peach aphid (*Myzus persicae*) and blackbean aphid (*Aphis fabae*) Aphid species were detected by Dr. Halgurd Rashed Ismael

(Specifc in insect taxonomy/ Department of Plant Protection/ College of Agriculture/ University of Duhok). A group of non-winged green peach aphids and blackbean aphids were collected from roses and beets in the fields of College of Agriculture/ University of Duhok with a soft brush and prepared a culture of each of the species by their breeding on healthy dahlia plants in cages under plastic house. After a month of breeding, five insects of each species were transferred and placed in plastic petri-dishes containing a slightly moistened filter sheet for an hour for starving. The starved insects were transferred to dahlia plants by the virus isolate and allowed to settle on their surface and begin probing to initiate virus acquisition for 5 minutes, and then transferred by five insects/ healthy dahlia plant containing 4-5 fully developed leaves (10 test plants for each type of aphid) and left to feed to initiate inoculation for 5 minutes. Plants were sprayed with an insecticide to kill insects, and inoculated plants were kept in cages inside the plastic house for a month to observe the symptoms (Eid and Pappu, 2014).

RESULTS AND DISCUSSION

Molecular Diagnosis of *Dahlia mosaic virus*

Infected and healthy dahlia plants were analyzed with three primer pairs specific for mosaic diseases that infect dahlia. DMV was detected in the leaves (+L) and tubers (+T) of infected dahlias by using primer pair of the DMV F1/R1. The primer pair when used in PCR consistently produced a PCR amplicon of expected size of about 900s bp from infected samples. No amplification could be seen in DNA from non-infected plant samples including leaves (-L) and tubers (-T) (Fig.1). There was no evidence of infection with either DMV-D10 or DCMV.

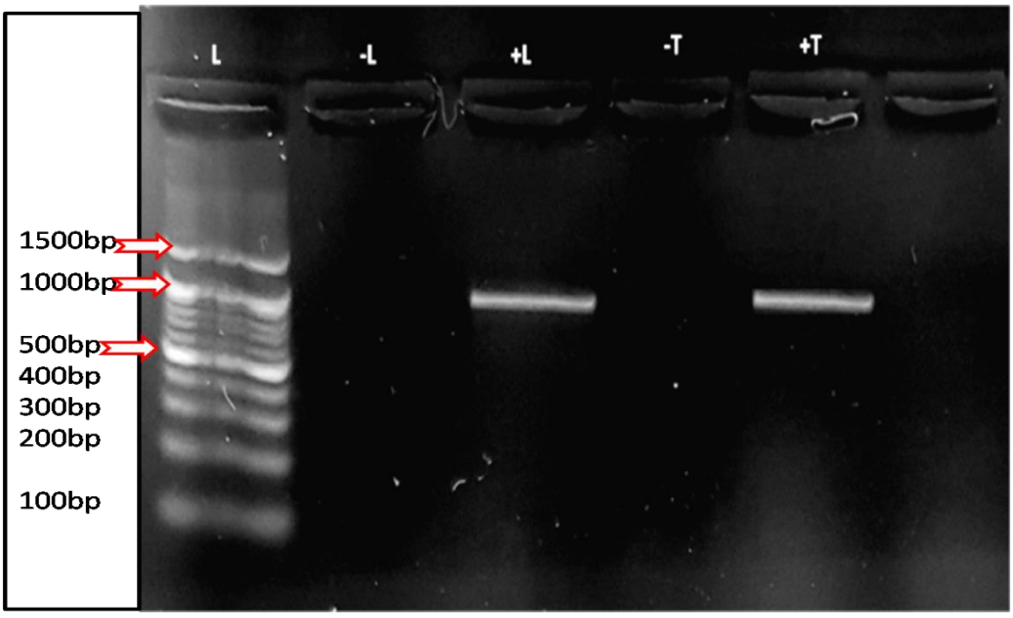

Fig. (1): Agarose gel electophoresis of polymerase chain reaction (PCR) products amplified with DMV F1/R1. L, 1-kb DNA ladder; -L Negative Leaf Sample; +L, Positive Leaf Sample; -T, Negative Tuber Sample; +T, Positive Tuber Sample.

PCR is a scientific protocol used to amplify, or create millions identical copies of a particular DNA sequence within a tiny reaction tube. Prior to the initiation of each new round for DNA amplification, the DNA strand is denatured and then two sets of oligonucleotides (primers) anneal to the denatured complementary strand. Then, by using DNA polymerase, primers lead DNA synthesis. PCR has been used as one of core techniques to molecular biology based-researches in a many of applications such as cloning, genotyping, sequencing, gene expression analysis, gene manipulation, and mutagenesis. PCR has also been used as a diagnostic protocol to detect plant diseases (Makkouk and Kumari, 2006; Schaad and Frederick, 2002). Recently, it is a popular technique for plant virus detection in the

laboratory and is very commonly used in molecular experiments (Webster *et al*., 2004). This technique is currently used as the basis of all diagnostic techniques (Lopez *et al*., 2008). PCR is able to process by the specificity of the primers (Makkouk and Kumari, 2006; McCartney *et al*., 2003).

Biological assays

Symptomatology. All of the examined indicator plants were responded successfully to viral inoculation mechanically by using phosphate buffer at 7.5pH, except *Chenopodium quinoa* and *Nicotiana tabacum*. The symptoms description on the inoculated plants is as follows. The symptoms appeared on 80% of the plants after three weeks as mottling, systemic vein clearing, vein banding, deformation of leaves and plant stunting (Fig. 2). No symptoms were observed on tubers of inoculated plants. The inoculated *Z. elegans*, also infected systemically and showed systemic leaf distortion and slight mottling and vein clearing (Fig. 3). The results also showed that *A. conyzoides* was infected with the virus isolate and showed chlorotic lesions and vein distortion after three weeks post inoculation (Fig. 4). Depending on the developed symptoms, *A. caudatus* responded locally to the virus isolate then followed by systemic infection. The symptoms were chlorotic local spots and ring spots on inoculated leaves followed by leaf distortion that appeared after 10 days from inoculation (Fig. 5). The virus isolate induced systemic infection in *H. annuus* as transient chlorosis followed by leaf crinkling (Fig. 6). Systemic transient chlorosis and slight mottling indicated that *C. indicum* were responded systemically to the virus isolate (Fig. 7). After several inoculation, no any symptoms observed on the inoculated *C. quinoa* and *N. tabacum*.

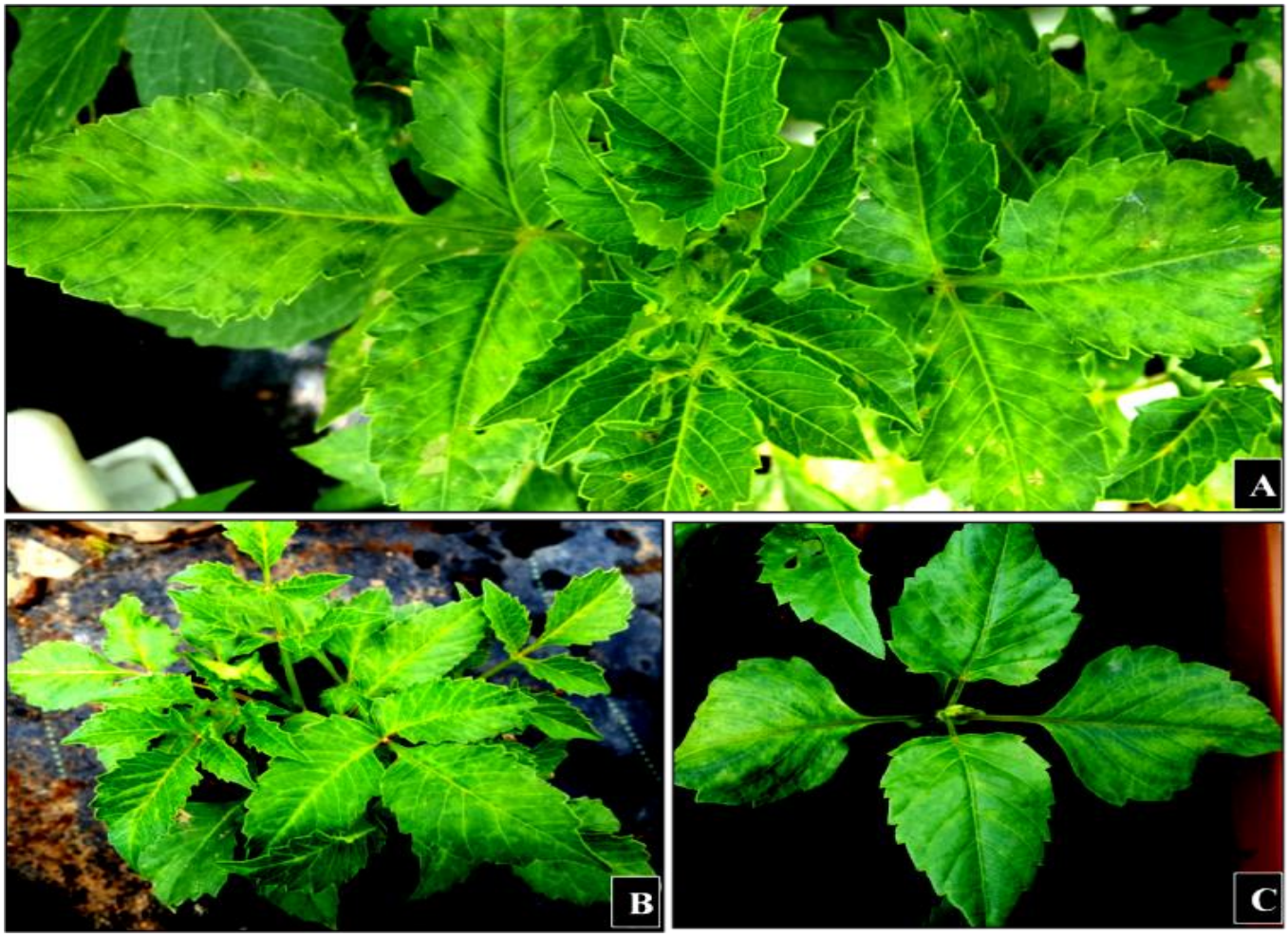

Fig. (2): Symptoms of DMV on dahlia plants. (A) Severe mottling, (B) vein clearing and leaf deformation, (C) vein banding.

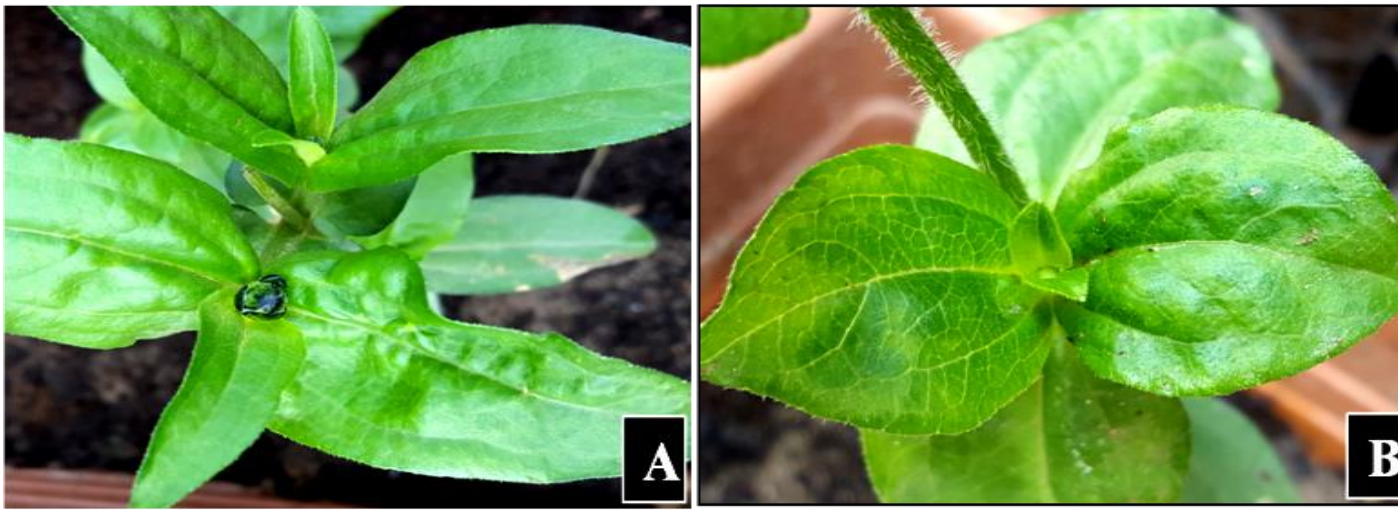

Fig. (3): Symptoms of DMV on *Z. elegans*. (A) Distorted leaves followed by (B) slight mottling and vein clearing.

Fig. (4): DMV Infected *A. conyzoides* with chlorotic lesions and vein distortion.

Fig.(5): Symptoms of DMV on *A. caudatus*. (A) local spots and ring spots and leaf distortion. (B) Clorotic local spots followed by leaf distortion.

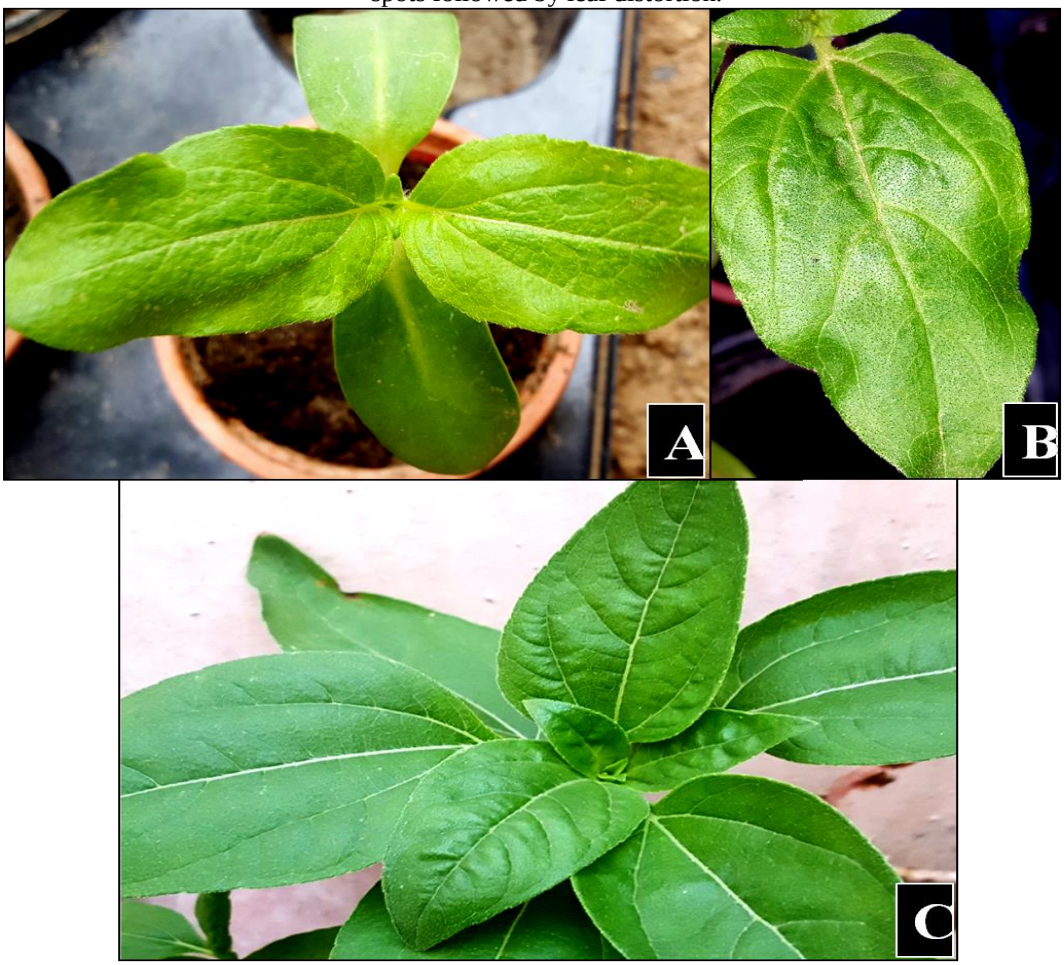

Fig. (6): *Dahlia mosaic virus* infected *Helianthus annuus*. (A) Transient chlorosis. (B) and (C) Leaf puckering and crinkling.

Fig. (7): *Dahlia mosaic virus* infected *Chrysanthemum indicum* showing transient chlorosis and slight mottling

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According to our results of symptomatology on indicator plants, DMV was diagnosed successfully in the surveyed fields of Duhok province. Disappearance of symptoms on inoculated *C. quinoa* and *N. tabacum* indicated the absence of *Cucumber mosaic virus* (CMV) (Ayo-John and Hughes, 2014), *Tomato spotted wilt virus* (TWSV) (French *et al.*, 2015), *Tobacco rattle virus* (TRV) (Hamed *et al.*, 2012), *Tobacco streak virus* (TSV) (Abtahi and Habibi, 2008) in infected dahlia plants (Brunt *et al*., 1996 and Jacobus *et al.*, 2017)**.** *Helianthus annuus* and *Chrysanthemum indicum* are the plants used in this study as indicator plants for the studied virus. Results showed that these two plants are new recorded hosts that responded systemically to DMV.

Transmission by aphids

Four weeks after inoculation of healthy dahlia plants by two species of aphids, *M. Persicae* and *A. fabae*, the specific symptoms of DMV were developed on all inoculated plants including vein clearing and slight mosaic (Fig. 10), the two used species were identified as vectors for DMV. These result were in agreement with Brunt (1971). Eid and Pappu (2014) found that the virus were transmitted by *M. persicae* at the rate of 50%. DMV is transmissible by 13 aphid species, notably *Aphis fabae* and *Myzus persicae*. All vectors' instars can transmit the virus but it does not pass through the eggs. Insect can acquire DMV by in one minute and inoculate new plant in five min. latent period has not been recorded. Both feeding and starved vector can retain the virus; therefore it differs from typical 'non-persistent' viruses (Brunt, 1971).

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