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Transmission Efficiency of Lettuce Mosaic Virus (LMV) by Different Aphid Species and New Aphid Vectors in Egypt

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Abstract: Seven different aphid species (Homoptera: Aphididae) were recorded on lettuce crops cultivated in the field at Giza region, Egypt, throughout 2010/2011 growing season. The most abundant species was the green peach aphid, *Myzus persicae* (Sulzer). On the other hand, 14 different aphid species (winged forms) were recorded in the yellow pan-water traps. The most dominant two aphid species were *M. persicae* followed by the lettuce aphid *Hypromyzus lactucae* (Lin.). Lettuce mosaic virus (LMV) was isolated from naturally infected lettuce plants grown at Giza region, Egypt. Infected lettuce plants having syndromes including, mosaic, mottling, leaf distortion and stunting, were collected and the causative agent was identified according to: symptomology, diagnostic hosts, serology (DAS-ELISA) and transmission tests (mechanical and aphids). LMV produced symptoms on 12 different diagonstic hosts; *Chenopodium quinoa, Chenopodium amaranticolor, Chenopodium murale, Chenopodium album, Lactuca sativa, Nicotiana tabacum, Nicotiana rustica, Datura innoxia, Gomophrina globosa, Sonchus oleracae, Cichorium endivia* and *Spinacia oleracea.* Sixteen different aphid species were tested as vectors of LMV; the most efficient vector was *Myzus persicae* (86%). Both *Acyrthosiphon lactucae* and *Acyrthosiphon kondi* recorded as vectors of LMV for the first time in Egypt.

Key words: Aphid vectors · Lettuce mosaic virus · Transmission · Efficiency

INTRODUCTION

The total area of lettuce and chicory in Egypt is about 5,000 feddan (one feddan = 0.42ha). Virus diseases recorded on lettuce include: Arabis mosaic virus (ArMV), Beet western yellows virus (BWYV), Cucumber mosaic virus (CMV), Lettuce big-vein virus (LBVV), Lettuce mosaic virus (LMV), Lettuce necrotic yellow virus (LNYV), Tobacco necrosis virus (TNV), Tomato spotted wilt virus (TSWV) and Turnip mosaic virus (TuMV) [1]. Lettuce mosaic virus (LMV) is a serious disease in commercial lettuce crops worldwide [2]. Lettuce plants Infected with (LMV) usually show vein clearing, yellow mottling, stunted growth and improper heading [3]. Also, it was isolated in Egypt by Fegla *et al*. [4] in Alexandria Governorate and Abdel-Aziz [5] isolated and characterized LMV from safflower plants in Minia Governorate. LMV is aphid-transmitted in a non-persistent manner by different aphid species (*e. g*. *A. gossypii, M. euphorbiae* and *M. persicae* [6] and is also seed-transmitted [7]. Several species of aphids, mostly *M. persicae* (Sulzer) and periodically *Macrosiphum euphorbiae* Thomas *Nasonovia ribisnigri* and *Pemphigus bursarius* infest lettuce in Egypt [8]. *H. lactucae*c also a vector was recorded on *Sonchus oleraceus* at Giza and North Sinai Governorates, during March and April. *Pemphigus bursarius* L. transmitted LMV but it was less efficient in transmitting the virus than *Myzus persicae* (Sulzer) [9]. In Egypt, Abd El-Aziz [5] reported 45% transmission of LMV by *Myzus persicae.*

The present study focuses on the different aphid species associated with lettuce crops and their relative efficiency as vectors of LMV in Egypt.

MATERIALS AND METHODS

Survey of different aphid species associated with lettuce plants in the field (located at the Agricultural Research and Experiment Station, Faculty of Agriculture Cairo University, Giza Governorate, Egypt) was made by direct counting method in which the total number of

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each aphid species (on the sample of 20 random plants) was counted. Also, a yellow pan-water trap described by Moericke [10] was used for monitoring the winged forms of aphid species. The traps were weekly inspected for the presence of aphids. Collected different aphid species were mounted, examined and identified according to the description of Claude and Guy [11].

Virus Detection: Detection of the causative agent (*i.e*. lettuce mosaic virus (LMV)), was based on the following methods: symptomatology, diagnostic hosts, serological reactions and both mechanical (sap) and aphid transmission tests.

Plant Sampling and Virus Identification: Regular field observation on lettuce plants in Giza Governorate revealed disordered plants showing signs of vein clearing, yellow mottling, stunting, chlorosis and mosaic. These plants were sampled then transferred to the laboratory for both sap (mechanical) and aphid inoculation tests as well as DAS- ELISA test to identify the causative disease agent. Collected Samples were kept separately at 4°C until DAS-ELISA was performed as described by Clark and Adams [12]. Samples were considered positive if the absorbance value at OD 405 nm was twice the average value of healthy controls.

Mechanical Inoculation: LMV infected-leaves were ground 1:2 (w/v) in 0.05 M potassium phosphate buffer, pH 7.0, containing 0.1% Na_2SO_3 and Carborundum (600) mesh was added before rub-inoculation on diagnostic hosts.

Diagnostic Hosts: *Lactuca sativa*, *Chenopodium quinoa*, *Ch. amranticolor*, *Ch. murale, Ch. album, Nicotiana tabacum, N. rustica*, *D. innoxia*, S. oleracae and *Gomphrena globosa*, at the three to five leaf stages were rub-inoculated with sap and maintained in an insect-free greenhouse [13, 14]. The presence of the virus was recognized based on the appearance of symptoms 2-3 weeks after inoculation and testing each plant by DAS-ELISA.

Aphid Transmission: Aphid transmission tests were carried out in the greenhouse using 16 different aphid species, *Acyrthosiphon lactucae,* Mordvilko, *A. pisum* (Harris), *A. kondoi* Shinji, *Aphis craccivora* Koch, *A. fabae* Scopoli, *A. gossypii* Glover, *A. nerii* Boyer, *Brevicoryne brassicae* (L.), *Dactynouts sonchi* (L.), *Hyperomyzus lactucae* (L.), *Macrosiphum euphorbiae* (Thomas), *Myzus persicae* (Sulzer), *Rhopalosiphum maidis* (Fitch), *R. padi* (L.), *Schizaphis graminum* (Rondani), *Therioaphis trifolii* (Monell). Under green house condition, the four aphid species: *A. craccivora, A. fabae, A. pisum* and, *A. kondoi* were maintained on faba bean healthy seedlings, while *A. gossypii* on *squash; M. persicae, M. euphorbiae* and *B. brassicae* on cabbage and *R. maidis* and *R. padi*, *S. graminum* were maintained on barley and wheat seedlings, while *D. sonchi, A. lactucae* and *H. lactucae* on sowthistle and lettuce seedlings.

Apterous and alate forms from each aphid species were tested after 5 min acquisition access time (AAT) on infected plant and groups of five aphid individuals were transferred to each test plant. These aphids were kept on the virus-free test plants under a glass cage for at least 2 hrs, as an inoculation access time (IAT). All tested plants were then sprayed with (Malathion 0.1%). Untreated healthy seedlings were used as control in each transmission test. Leaf samples gathered from all treated plants were tested for the presence of LMV using double-antibody-sandwich enzyme-linked immunosorbent assay (DAS-ELISA).

RESULTS

Seven different aphid species were associated with lettuce plants in the field they were; *H. lactucae*, *M. euphorbiae*, *M. pericae*, *A. lactucae*, *D. sonchi*, *A. gossypii* and *A. pisum. M. persicae* and *A. lactucae* were the most abundant species recording 70.6 and 52.6 individual/plant, respectively, followed by *H. lactucae* and *M. euphorbiae,* whereas, *A. pisum* was the least abundant recording 15.5 individual/plant) (Fig. 1). The average number of winged forms of different aphid species recorded per yellow pan water trap during 2011/2012 season (Fig. 2) revealed the occurrence of 14 different aphid species. These species were; *A. craccivora, M. persicae, A. nerii, A. pisum, B. brassicae, D. sonchi, H. lactucae*, A. gossypii, *R. maidis*, *R. padi*, *S. graminum*, *A. lactucae*, *T. trifolii* and *A. kondi.* The most dominant winged aphid species was *M. persicae* with an average number of 9.1 individuals/trap followed by *H. lactucae* (8.6 individual/trap) and *S. graminum* was the least dominant aphid species.

Diagnostic Hosts and Symptomatology: The obtained results show that, LMV produced different visible symptoms including: mosaic, leaf distortion, vein clearing and dwarfing on lettuce plants as shown in Fig. 3 a, b & c. *N. tabacum, N. rustica* and *D. innoxia*

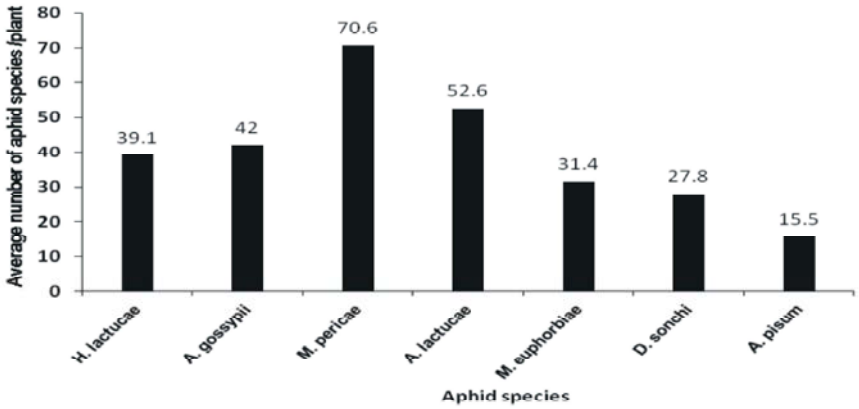

Fig. 1: Average number of 7 different aphid species recorded lettuce plant in the field (Giza, 2011/2012)

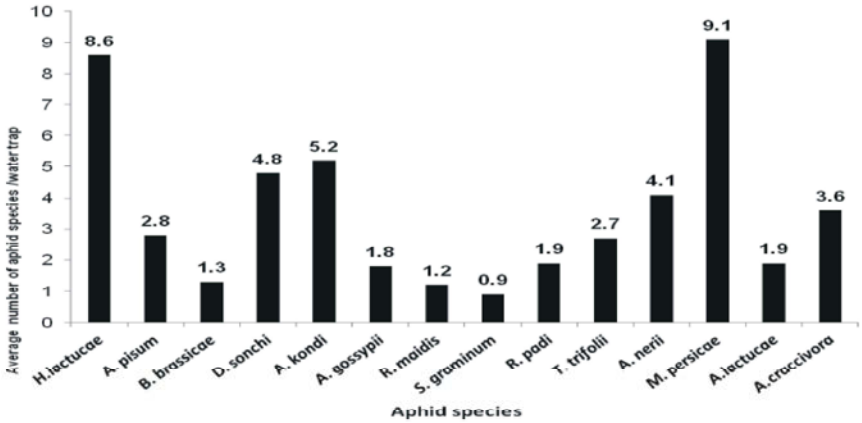

Fig. 2: Average number of 14 different aphid species recorded per yellow water trap located in the field (Giza, 2011/2012)

developed systemic mosaic (Figs 4 & 5). While, *Ch. quinoa, Ch. amaranticolor, Ch. murale* and *Ch. album* plants developed chlorotic local lesions on inoculated leaves 4 to 7 days after inoculation and systemic symptoms which included chlorotic lesion and leaf distortion 2 weeks after inoculation. *Ch. quinoa* was more sensitive than *Ch. amaranticolor as* local lesions were more numerous but without reddish margins (Figs.6 &7). *G. globosa* developed necrotic local lesions 2 to 3 weeks after inoculation (Fig. 8).

Serological Test: Twelve tested LMV-infected host plants listed in Table 1 were reacted positively to LMV antisera; using ELIZA test.

Insect Transmission Tests: In the present study the 16 different aphid species, listed in Table 2 including, blue clover aphid, *Acyrthosiphon kondoi* Shinji, which is for the first time recorded in Egypt (unpublished data), were tested as vectors of lettuce mosaic virus (LMV).

Table 1: The positive reaction of the diagnostic hosts to LMV antisra

No	Diagnostic Host	*Syndromes	ELISA Read (Mean)	
1	*Chenopodium quinoa*	vc, sl, ld, chll	0.244	+++
2	*Ch. amaranticolor*	vc, sl, chll	0.213	+++
3	*Ch. murale*	vc, s, m, ld, dh	0.267	+++
4	*Ch. album*	vc, sl, chll	0.253	+++
5	*Lactuca sativa*	vc, m, d,dh, ld,s	0.284	++++
6	*Nicotiana tabacum*	M	0.246	+++
7	*N.rustica*	M	0.249	+++
8	*Gomphrena globosa*	Nll	0.222	+++
9	*Sounchus oleracae*	vc, m, chll	0.195	++
10	*D.innoxa*	M	0.199	++
11	*Cichorium endivia*	vc, m,	0.186	++
12	*Spinacia oleracea*	Chll	0.195	++
13	Control	-	0.112	-

*vc: vein clearing chll: chlorotic local lesion s: stunting

sl: systemic lesion m: mosaic d: dwarfing

ld: leaf distortion dh: defective heading nll: necrotic local lesion.

Fig. (3a): Symptoms of LMV on *Lactuca sativa*; includes; Vein clearing, mosaic, yellowing leaf distortion and stunting, necrotic spots may occur.

Fig. (3b): Symptoms of LMV on *Lactuca sativa*; includes; leaf distortion and stunting, necrotic spots may occur.

Fig. (3c): Lettuce leaves showing LMV symptoms; Vein clearing, mosaic, yellowing leaf distortion

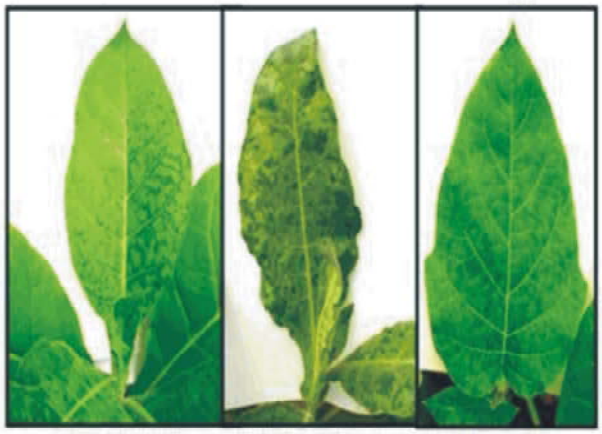

Fig. (4):*Nicotiana tabacum, N.rustica* systemic mosaic.

Fig. (5):*Datura innoxia* systemic mosaic and malformation

Fig. (6): *Ch. quinoa* and *Ch. album*, - necrotic local lesions; then systemic mosaic.

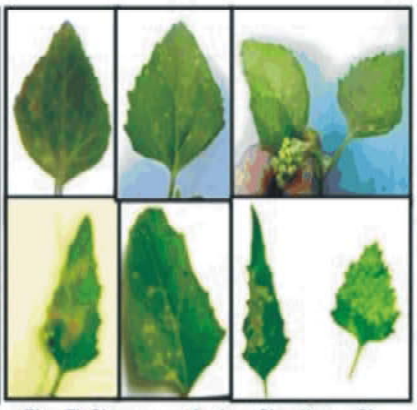

Fig. (7):*Ch. amaranticolor, Ch. album, Ch. quinoa* and *Ch.murale* showing necrotic local lesions; then systemic mosaic.

Fig. (8):*G. globosa* showing necrotic local lesions; not systemic.

Table 2: Percentage of transmission of LMV by 16 different species and forms of aphids.

No.	Aphid species	% of LMV transmission		
		Wingless form	Winged form	Total
1	*Acyrthosiphon lactucae*	4/22(18.2%)	2/28(7.1)	25.3
2	*Acyrthosiphon pisum*	2 /28(7.1%)	1/18(5.6%)	13.7
3	*Acyrthosiphon kondoi*	3/28(11%)	1/13(8%)	19.0
4	*Aphis craccivora*	4/29 (13.8%)	1/16(6.2%)	20.0
5	*Aphis fabae*	2/27(7.4%)	1/16(6.2%)	13.6
6	*Aphis gossypii*	9/29(31%)	2/29 (7%)	38.0
7	*Aphis nerii*	3/27 (11%)	1/19(5.3%)	16.3
8	*Brevicoryne brassicae*	2/26 (8%)	0/18 (0%)	8.0
9	*Dactynouts sonchi*	3/26(11.5%)	1/17(6%)	17.5
10	*Hyperomyzus lactucae*	11/30(36.7%)	1/18(5.6%)	42.3
11	*Macrosiphum euphorbiae*	4/28(14.3%)	2/19(10.2%)	24.5
12	*Myzus persicae*	19/28 (67.8%)	5/28(18%)	86.0
13	*Rhopalosiphum maidis*	1/22(4.5%)	0/15(0%)	4.5
14	*Rhopalosiphum padi*	1/28 (3.6%)	0/14 (0%)	3.6
15	*Therioaphis trifolii*	0/23(0%)	0/14(0%)	0.0
16	*Schizaphis graminum*	0/25(0%)	0/13(0%)	0.0

The results revealed that the most efficient vector was *M. persicae* (86.0%) followed by *H. lactucae* (42.3%), *A. gossypii* 38.0% then *A. lactucae* 25.3% and *M. lactucae* 24.5%. The other species were less efficient vectors. Whereas, *T. trifolii* and *S. graminum* were not able to transmit the virus. The alate (winged form) aphids of *M. persicae* were more efficient vectors of LMV than the other wingless forms. In the present study, both *A. lactucae* and *A. kondi* are recorded as vectors of LMV for the first time in Egypt.

DISCUSSION

As with other potyviruses LMV is transmitted efficiently by aphids in non persistent manner [9]. In the present study fourteen different aphid species were surveyed using water pan-traps on lettuce plants in the field. The main dominant aphid species colonizing on lettuce plants was *M. persicae;* this finding are in agreement with those obtained by Nebreda *et al.* [15], who reported that the Moericke traps generally caught more aphid species than the tile trap in lettuce and broccoli fields, they added that, the main aphid species colonizing lettuce was *Nasonovia ribisnigri*, but other less abundant colonizing species were *Aulacorthum solani* and *Macrosiphum euphorbiae.* The most efficient vectors of LMV were *M. persicae, Aphis gossypii*, *H. lactucae,* while both *A. fabae* and *M. euphorbiae* transmitted the virus with low efficiency, *Rhopalosiphum padi* and *N. ribisnigri* did not transmit the virus, as previously stated by Nebreda *et al.* [16]. The spread of viruses transmitted in a non-persistent manner by aphids often occurs when non-colonizing species land on the crop which is host to the virus. The low abundance of some aphid vector species, therefore, does not mean their less importance in the dispersal of the disease. The most abundant aphid species, on the plants are also the most abundant species caught in the traps [17].Therefore, using water traps to determine the different aphid species landing on Lettuce is a reliable parameter to predict the presence of LMV vectors. Also, vector species found colonizing lettuce and those caught by traps together play important role as vectors of LMV in the field [18, 19].

CONCLUSION

LMV transmitted by different aphid species, in a non persistent manner. The control of LMV, relied on prophylactic measures such as the use of virus free seeds, vector aphids control in order that keep insect population low in the fields, elimination of alternative hosts.

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