Egyptian J. Virol., Vol. 9:110-115, 2012

Effect of environmental factors on Peanut mottle virus infection and population of insects on Lentil in Egypt

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ABSTRACT

Peanut mottle virus (PMoV) was isolated from Lentil *cvs.* Giza 51. The infected plants feature severe mosaic on Lentil *cv.* Giza51, mottling on *cv.* Giza9, mild mottle on *cv.* Giza 4, yellow mild mottling on *cv.* Giza370. Seed transmission tests of PMoV confirmed of the virus transmission through Lentil seeds. *Peanut mottle virus* was detected in the testas, cotyledons and embryo dissected from seeds of each Lentil cultivates. Electron microscopy of PMoV showed virus particles aggregates and degenerated mitochondria in cytoplasm of lentil leaf cells infected with PMoV. Effect of environmental factors and populations of insects on virus infection revealed a positive relationship was recorded between insect incidence and percentage of virus infection during 2007 and 2009, meanwhile the temperature and relative humidity were lower in Behara than Fayuom, the percentage of virus infection were higher.

KEY WORDS: PMoV, seed transmission, infected seed, electron microscopy, environmental factors.

INTRODUCTION

Lentil crop is one of the most important crops in Egypt. Being the main source of plant protein for human compared with expensive meal of meat or chicken. Faba, bean, lentil and chick pox are often infected by several viruses which cause a reduction in the yield as will as the quality of the crop (Allam *et al.*, 1979; Makkouk *et al.*, 1992 and Salama, 1998). *Peanut mottle virus* (PMoV) is a member of the genus potyvirus which cause the economically important legume diseases. It is spread in African region, the east Asian region, Colombia. India, Japan, Malaysia, Philippines, Taiwan and USA (the south east), (Nischwitz *et al.*, 2007).

Seed transmission on of PMoV and some epidemic causing factors *i.e.* population of aphid vectors in relation to virus infection (Madden *et al.*, 2000).

This study aims to isolation PMoV from naturally infected and confirm the virus exptence by serologically and Electron microscope examination.

MATERIALS AND METHODS

In order to evaluate the incidence of PMoV infected lentil in Egypt, naturally infected lentil plants Giza51 were collected during two successive seasons (2007/08, 2008/09) from Fayuom and Behara Governorates.

Virus detection

Naturally infected lentil plants *cv.* Giza51 revealed viral symptoms (mosaic, yeliowing, stunting and mottling) were collected from commercial fields at Fayuom Governorate. Samples were serologically checked against PMoV by indirect-ELISA. The virus isolate was mechanically inoculated in Lentil *cvs.* Giza9, Giza4, Giza51, Giza370 and kept under green house conditions.

Seed transmission

To study the PMoV transmission through lentil seeds, two hundred lentil seeds *cvs.* (Giza9, Giza4, Giza51 and Giza370) collected from previously inoculated lentil were sown in sterilized pots 20Q and kept in insect proof greenhouse for symptoms observation till four weeks after sowing. The percentage of virus transmission through seeds were determined using Indirect-ELISA.

Presence of PMoV in seed parts

The lentil seeds were soaked in distilled water on cellulose paper for overnight and continuous light and carefully dissected into their components. Testa, Cotyledon and embryo. The identity of the virus isolate was confirmed by specific double-antibody sandwich enzyme-linked immuno-sorbent assay by **Clark and Adams (1977)** were purchased from Sanofi, Sante Animal, Paris, France.

Electron Microscopic Examination (EM)

The carbon-coated grids were floated on drops. The grids were stained with 2% uranylacetate for 2 min. and air dried. Grids were examined under SEO (Sunny Electron Optics) TEM-100 at electron microscopy unit, VACSERA, Egypt.

Effect of environmental factors (Temperature and Relative Humidity %) on virus infection:

Virus-free colonies of *Myzus persicae* and *Aphis craccivora* were allowed to acquire (PMoV) from PMoV sources of infection (Fayuom). Previous aphid species were allowed to inculcate healthy Lentil seedlings to determine virus-transmission ability adult age of each *M. persica* and *A. craccivora*. Under Field conditions, some experiments were carried out in two different locations namely Behara and Fayuom Governorates, during the growing season of 2007/08 and 2008/09. The symptoms were recorded weekly for each lentil *cv.* Giza51 under field conditions in relation with temperature to humidity, and the relation between the severity of symptoms and populations of insects and environmental conditions were considered. Every fifty plants were taken from the bottom, the middle and the top plants monthly. Five places were carried out randomly to calculate the percentage of infection of this feddan. The two species were collected and identified in Department of Economic Entomology and pesticides, Faculty of Agriculture, Cairo University.

RESULTS AND DISCUSSION

Detection of PMoV

Naturally infected lentil plants showed yellow mild mottling symptoms, (Fig.1) gave+ve results by indirect ELISA against PMoV.

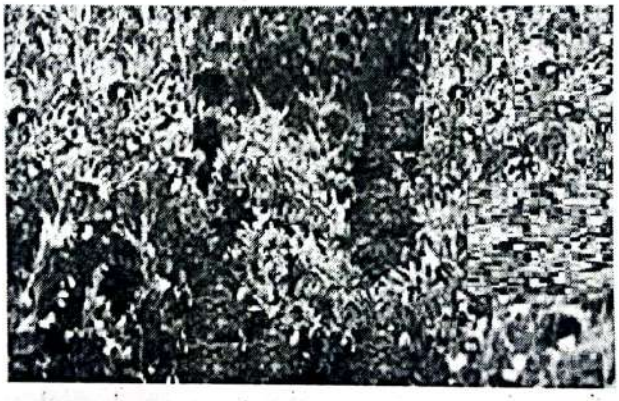

Fig. 1. The Naturally infected lentil plants *cv.*Giza51,appeared symptoms of PMoV (yellow, mottling and stunting) in open field

Seed transmission

Twenty two Lentil seeds were taken from infected plants. Results in Table (1) indicated that PMoV was transmitted through lentil seeds. The percentage of seed transmission differs according to cultivar. PMoV was transmitted through 22%, 15%, 13% and 5.5% in Giza9, Giza4, Giza51 and Giza370, respectively. These results were confirmed using Indirect ELISA. This results were agreement with **Crowley (1959).**

Presence of PMoV in seed parts.

The virus isolate was detected in the testas, cotyledons and embryo of infected seeds. The virus was present at a higher proportion of testas and cotyledons of each cultivars while was present a lowest percentages only in the embryos of cultivars Giza9 and Giza51, in which the percentages were 20%, 15% respectively, on the contrary, no virus was detected in both of Giza4 & Giza370 embryo (Table 2).

Table 1. Percentages of seed transmission of PMoV in different Lentil cultivar.

Cultivar	No. of tested seeds	No. of infected seeds	% of seed transmission
Giza9	200	44	22
Giza4	200	30	15
Giza51	200	26	13
Giza370	200	11	5.5

Table 2. Detection of PMoV in different parts of Giza4, Giza370, Giza9 and Giza51 lentil seeds.

Cultivars	No.of infected Testa	Persentage	Cotyledon	Persentage	Embryo	Persentag
Giza4	6/20	30.0%	6/20	30.0%	0/20	0.0%
Giza370	5/20	25.0%	1/20	5.0%	0/20	0.0%
Giza9	12/20	60.0%	6/20	30.0%	4/20	20.0%
Giza51	11/20	55.0%	6/20	30.0%	3/20	15.0%

Table 3. Response of Lentil cultivars to PMoV infection.

Cultivars	% PMoV infection	% Disease severity	Cultivars response
Giza9	25	55	Susceptible
Giza4	15	32	Susceptible
Giza51	13	30	Resistant
Giza370	0.055	0.11	Tolerant

- Each value are mean of five replicates.

The cultivars of Lentil Giza 9, 4, 51 and 370 were revealed differentiation of virus infection response (Table 3). Lentil Giza 370 cultivar appear tolerant against PMoV and low disease severity. Giza 51 cultivar showed virus resistant with 13% and disease severity with 30% on the other hand cultivar Giza 9 appear virus susceptible with 25% and disease severity 55% as a susceptible cultivar.

Electron microscopic examination of PMoV

Twenty-five days after mechanical inoculation young leaves of Giza51 seedlings showing curl and symptoms, were examined by Electron microscope showing filamentous virions not enveloped usually flexuous with a clear modal length of 740-750 nm (but may rang between 704-984), several investigators reported similar results **(Xu, 1984)**. The inclusion bodies has been observed which family poty-virus consequently confirm the virus existence

Effect of temperature and relative humidity on virus infection.

Behara and Fayuom Governorates were chosen for different weather conditions (obtained from Forecast and Climatic Unit. Agric. Research Center), during growing season 2007/08 and 2008/09. The temperature in Behara ranged between 17 to 27°C the relative humidity ranged between 44 to 63%, the percentage of virus infection under the above mentioned conditions was between 5 to 30% (Table, 5).

In the second location Fayoum, the temperature was ranged between 18 to 31°C the relative humidity ranged between 45 to 66 %, the percentage of virus infection under the above mentioned conditions was between 10% to 75%, Table (4).

In general during 2007 and 2009 the temperature and relative humidity were lower in Behara than Fayuom. The percentage of virus infection in Behara and Fayuom were higher in APR during 2007/08 and 2008/09.The percentage of virus infection was higher in Faym than Behara, Table (6 and 7).

The present experiments were carried out to study the relationships between environmental conditions and the insect vector including aphid during the whole growing

season, their distribution on the same plants, their ability to transmit certain viruses as well as the impact of the insect population density on the virus infection. The experiments were carried out under field conditions in Behara and Fayum, during the 2007/8 and 2008/9.

Table 4. Relation between the virus infections, populations of insects and environmental conditions for Lentil plants (*cv*.Giza51) during the growing season of 2007/08 in Fayuom Governorate.

Month	Populations of insects in per 50 plants						Temp °C	Hum %	% infected plants
	Myzus persicae			*Aphis craccivora*					
	T	M	B	T	M	B			
DEC	95	0	0	81	0	0	22	64	10
JAN	525	3	0	335	1	0	18	45.6	15
FEP	575	7	2	405	25	10	20	50	18
MAR	1970	25	15	575	110	35	24	46.7	20
APR	3890	105	45	750	175	55	31	47.8	42

T: Top of plant M: Middle of plant B: Bottom of plant Temp.= Temperature Hum.=Humidity

Table 5. Relation between the virus infections, populations of insects and environmental conditions for Lentil plants (*cv*.Giza51) during the growing season of 2007/08 in Behara Governorate.

Month	Populations of insects in per 50 plants						Temp °C	Hum %	% infected plants
	Myzus persicae			*Aphis craccivora*					
	T	M	B	T	M	B			
DEC	75	0	0	60	0	0	20	59	5
JAN	370	5	0	210	3	0	18	44	6
FEP	425	9	0	325	5	0	17	46	10
MAR	875	15	10	950	25	15	22	46	15
APR	1110	85	35	1070	70	20	27	47	21

T: Top of plant M: Middle of plant B: Bottom of plant Temp.= Temperature Hum.=Humidity

Table 6. Relation between the virus infections, populations of insects and environmental conditions for Lentil plants (*cv*.Giza51) during the growing season of 2008/09 in Fayuom Governorate.

Month	Populations of insects in per 50 plants						Temp °C	Hum %	% infected plants
	Myzus persicae			*Aphis craccivora*					
	T	M	B	T	M	B			
DEC	60	0	0	61	0	0	24	66	10
JAN	330	2	0	210	3	1	21.8	51.7	15
FEP	420	5	3	325	15	5	23.1	53.6	18
MAR	1450	30	10	1220	75	10	26.3	55.6	35
APR	4920	75	25	1300	90	20	29.4	54.8	75

T: Top of plant M: Middle of plant B: Bottom of plant Temp.= Temperature Hum.=Humidity

Table 7. Relation between the virus infections, populations of insects and environmental conditions for Lentil plants (*cv*.Giza51) during the growing season of 2008/09 in Behara Governorate.

Month	Populations of insects in per 50 plants						Temp °C	Hum %	% infected plants
	Myzus persicae			*Aphis craccivora*					
	T	M	B	T	M	B			
DEC	35	0	0	31	0	0	21	63	7
JAN	220	3	0	133	0	0	18	49	9
FEP	325	5	5	250	7	3	17	50.1	12
MAR	1230	13	6	915	30	5	21	51.3	21
APR	1350	35	15	1175	65	20	23	53	30

T: Top of plant M: Middle of plant B: Bottom of plant Temp.= Temperature Hum.=Humidity

Table 8. Determination of *Peanut mottle virus* transmission ability by *M. persica* and *A. craccivora*.

Exp. No.	*M. persica*	*A. craccivora*
1	19/20	12/20
2	17/20	14/20
3	15/20	10/20
%	85%	60%

In Behara, a positive relationship was recorded between insect incidence and percentage of virus infection. In the first season (2007-2008) the lowest infection percentage was 21, in the second season (2008/09) highest infection percentage was 30, Table (5 and 7). The degrees of temperature and relative humidity in season 2007/08 were 20°C, 18°C, 17°C, 22°C and 27°C and 59%, 44%, 465, 46%, and 47% in DEC., JAN., FEP., MAR., and APR. respectively, while during season 2008/09 were 21°C, 18°C, 17°C, 21°C and 23°C and 63%, 49%, 50%, 51%, and 53% in DEC., JAN., FEP., MAR., and APR. respectively (Table 5 and 7).

In Fayuom, a positive relationship was recorded between insect incidence and percentage of virus infection. In the first season (2007/08) the lowest infection percentage was 42, in the second season (2008/09) highest infection percentage was 75.

The degrees of temperature and relative humidity in season 2007-2008 were 22°C, 18°C, 20°C, 24°C and 31°C and 64%, 45%, 50%, 47%, and 48% in DEC., JAN., FEP., MAR., and APR. respectively, while during season 2008-2009 were 24°C, 22°C, 23°C, 26°C and 29°C and 66%, 52%, 54%, 56%, and 55% in DEC., JAN., FEP., MAR., and APR. respectively (Table 4 and 6).

These results were confirmed with Kerry (1980) that helps us in studding virus-vector relation ship for infection production. Both previously mentioned four aphid species are vectors for mosaic causing viruses fluctuation of FBNYV-infection rate and population fluctuation of its aphid vectors **(Abou-Alata *et al.*, 2005).**

Recorded data revealed that the incidence of *Myzus persicae* more than *Aphis ceaccivora* in both seasons.

The percentages of viral infection in Fuom Governorate were higher than in Behara Governorate in both years (2007/08 and 2008/09).

The virus could be transmitted by two different aphid species *i.e A. craccivora* was 60% and M. persica was 85%.

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