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EFFICIENCY OF TRANSMISSION PEA ENATION MOSAIC VIRUS (PEMV) BY THE PEA APHID IN DAKHLIA GOVERNORATE, EGYPT

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ABSTRACT

The pea aphid Acyrthosiphon pisum (Harris) is considered as vector for numerous plant viruses, including Pea enation mosaic virus (PEMV)). Experiments confirmed the ability of A. pisum adult to acquire and transmit the virus to pea plantations in Dakhlia Governorate, Egypt.

The acquisition threshold feeding period ranged 1-20 min from infected pea plant to healthy celery and from infected celery plant to healthy one 15– 25 min. Moreover retransmission from infected celery plants to healthy pea plants 10 - 20 min. On the other hand inoculation threshold feeding period ranged from15-25 sec. The incubation period in A. pisum was 4-6 hours but in pea plants ranged from 8-11 days and in celery plants 7--9 days. The Retention period of Pea enation mosaic virus (PEMV) in A. pisum was 4 - 5 days)

Results of this study will help to understand the epidemiology of Pea enation mosaic virus (PEMV)) in pea plantations .Also the pea plants and virus vector relationships of Daklia isolate are reported for the first time.

***Conclusively,** it can be concluded that transmission experiments proved that pea enation mosaic virus is transmitted by A. pisum in a persistent manner.*

Key words: *Acyrthosiphon pisum,* Pea enation mosaic virus (PEMV)s, aphid vector disease, pea plants diseases.

INTRODUCTION:

Pea (*pisum sativum*) which is commonly known in Egypt as “Besela” is a tender annual winter crop .It one of the most important vegetable crops in Egypt which is consumed as a cooked green seeds. Peas is full of nutrition because its grain is cheap source and rich in protein content (27.8%), as well as, complex carbohydrates (42.65%), vitamins, minerals dietary fibers and antioxidant compounds. Also, it has been shown to possess antibacterial, antidiabetic, antifungal, anti-inflammatory, anti-hypercholesterolemia, and antioxidant activities and also shown anticancer property. Its nonnutritive biologically active components include alkaloids, flavonoids, glycosides, isoflavones, phenols, phytosterols, phytic acid, protease inhibitors, saponins, and tannins (Urbano *et al*., 2003, and Dan, 2020).

The Pea enation mosaic virus (PEMV) is a virus infecting legume crops such as pea (*Pisum sativum*), lentil (*Lens culinaris*), chickpea (*Cicer arietinum*) and faba bean (*Vicia faba*). It causes various symptoms of infection, including the typical vein enations, plant growth reduction and pod and seed deformities that can lead to significant yield losses. The virus is transmitted in a circulative manner by aphids, mainly by the pea aphid *(Acyrthosyphon pisum*), and by mechanical inoculation, but the seed transmission was not observed (Timmermann-Vaughan *et al.*, 2009).

Acyrthosiphon pisum is a serious concern for commercial pulse producers because it can injure the crops directly by removing sap from leaves, stems, and pods, and indirectly by acting as a vector for over 30 plant viruses, including cucumber mosaic virus, beet yellows virus, pea enation mosaic virus, and bean leaf roll virus (Paudel *et al*., 2018,).

The pea aphid mainly attacks stems, terminal shoots, and petioles of seedlings and, as the plant matures, it attacks flowers and pods. *Acyrthosiphon pisum* infests several host plants and causes chlorotic damage with curling and wilting of leaves, nutrient deficiencies, and plant stunting. Furthermore, by puncturing cells with their needle-shaped stylus, these aphids cause an indirect and serious damage by injecting saliva that is phytotoxic to plants (Wang *et al*., 2017).

In addition, sooty mold develops on the host plant's leaves due to the aphid honeydew, which impairs photosynthesis (Lu and Kuo, 2008).

Maximum yield losses of up to 35.7% have been reported from A. *pisum* on pea in India (Bhatnagar, 1996).

In addition to Infected pea *(Pisum sativum* L.) plants develop a slight downward rolling of the trifoliate leaves 4-6 days post inoculation followed by a distinct yellow mosaic on the leaves. Also, it causing stunting and deformation of the plant and mottling and curling of leaves, and the disease can result in severe crop losses (c. 50%) in beans and peas (De Zoeten and Skaf, 2001).

On the other hand the epidemiology information of this disease is still lacking in Egypt.

Therefore, the scope of the present study was to contribute towards a better knowledge of virus-vector relationship.

MATERIALS AND METHODS

1 Source of the virus and its identification

Pea plant samples taken from pea plantation in pea plantation at Met Ghamer region , Dakhlia Governorate, Egypt a showing typical symptoms of Pea enation mosaic virus (PEMV) selected for virus isolation and identification .This symptoms showed causes vein clearing and the formation of translucent spots, which are apparent when infected leaves are held up to the light. Development of stipules (the leaf-like structures at the base of leaves) is often retarded, while leaflets become crinkled and may contain necrotic spots. Plant tops often become yellow and mottled, with distorted leaves. Pods may be severely malformed and fail to fill. At an advanced stage of infection, scaly leaf-like structures (enations) may appear .On the other hand some infected pea plant samples send to department of virology of plant pathology Institute agriculture research center(ARC),Cairo, Egypt to isolate the virus from infected samples.

2. ***Rearing the pea aphid Acyrthosiphon pisum (Harris*****)**

Adults of pea aphid were obtained from healthy pea plants and reared on cages pea plants *(Pisum sativum L.)* and kept the plants until finished of experiments to be sure it's free from any contaminating pathogens before using in tests.

3.***Test plants***

Two types of test plants were used in experiments:

A- The first healthy seedling pea plants as principle host of the virus.

B- The second was celery (*Apium graveolens*) plants as indicator for plant viruses.

4. Transmission experiments:

The experiments of virus transmission were conducted in the laboratory of Plant Production Department, Faculty of Technology and Development, Zagazig University, Egypt.

Aphid *Acyrthosiphon pisum* adult specimens collected from healthy pea plants were critically examined to be free from any contaminating agent before using in the test by placing them directly after collection from field on healthy faba bean *(Vicia faba*) for 14 days. The tested plants were kept under observation in the laboratory from disease symptoms. It is confirmed these adult insects used in feeding were free from disease pathogen. Healthy aphid insects were reared in cages in a rear room with 24 hrs light (El-sharkawy, 1989 & 2002).

Micro-isolators of the plastic leaf cages (1.5cm in length and 2.5cm in diameter), the top leaf cage covered by nylon-screen to allow the insect ventilation, were especially constructed to ensure the continues stay of the aphid on the host plant throughout the periods of acquisition and inoculated feeding (El-sharkawy, 2002)

The inspected aphid insects were classified into different groups according to the length
of acquisition feeding periods on infected plants with Pea enation mosaic virus disease. The aphid insets were starved for 1hr.befor using in the experimental transmission.

In both acquisition and inoculation feeding period, used a group of 10 aphid were transferred by a small brush to the plastic leaf cage, and placed on each plant. Aphid s were transferred daily to new healthy plant. The plants were kept under observation in laboratory for symptoms development .Subsequent transmission was also carried out to confirm the ability of tested Aphid *Acyrthosiphon pisum* insects to transmit the Pea enation mosaic virus.

The procedure for determining acquisition feeding periods (AP_{50}) and inoculated feeding period (IP_{50}) values were the same as described by (Tsai and Zitter, 1982). The experimental transmission replicated two times.

RESULTS AND DISCUSSION

1. *Transmission of Pea enation mosaic virus (PEMV) by the pea aphid (A. pisum):*

1.1. Effect of length of acquisition access period on the efficiency of (PEMV) transmission:

The results of primary experiments showed that the efficiency of PEMV transmission by the pea aphid *Acyrthosiphon pisum* increased gradually as acquisition access period (AAP) increased from infected pea plant to healthy celery plant and from infected celery plant to healthy plant and from infected celery to healthy pea plants (Tables 1, 2 and 3). The obtained results showed that efficiency of PEMV transmission by pea aphid ranged from 66.6 - 100 % , also the results obtained showed *A. pisum* in most cases can acquire viruses . It was determined that the pea aphid required the following minimum acquisition feeding periods from infected pea plant to healthy celery 1-20 min , from infected celery plant to healthy one 10 – 20 min . Also retransmission from infected celery plants to healthy pea plants occurred in10 - 20 min for Pea enation mosaic virus (PEMV).

Table (1): Effect of Acquisition access period 0f aphid vector A. *pisum* on transmission efficiency of Pea enation mosaic virus (PEMV) from infected pea plants to healthy celery plants.

Acquisition access period on infected pea plants (Min)	Total number of healthy celery plants infected	Efficiency of virus transmission, %
1	1/15	6.66%
5	1/15	6.66%
10	1/15	6.66%
15	1/15	6.66%
20	3/15	20%
25	4/15	26.66%
30	4/15	26.66%
35	7/15	46.66%
40	10/15	66.66%
45	11/15	73.33%
50	11/15	73.33%
55	13/15	86.66%
60	10/15	66.66%

Table (2): Effect of Acquisition access period 0f aphid vector *Acyrthosiphon pisum* on transmission efficiency of Pea enation mosaic virus (PEMV) from infected celery plants to healthy one.

Acquisition access period on infected celery plants (Min..)	Total number of healthy celery plants infected	Efficiency of virus transmission, %
1	00/15	00%
5	00/15	00%
10	00/15	00%
15	1/15	6.66%
20	1/15	6.66%
25	2/15	13.33%
30	3/15	20%
35	3/15	20%
40	5/15	33.33%
45	8/15	53.33%
50	12/15	80%
55	13/15	86.66%
60	11/15	73.33%

Table (3): Effect of Acquisition access period 0f aphid vector *Acyrthosiphon pisum* on retransmission efficiency of Pea enation mosaic virus (PEMV) from infected celery plants to healthy pea plants.

Acquisition access period on infected celery plants (Min.)	**` Total number of healthy pea plants infected**	**Efficiency of virus retransmission, %**
1	00/15	00%
5	00/15	00%
10	1/15	6.66%
15	1/15	6.66%
20	3/15	20%
25	4/15	26.66%
30	7/15	46.66%
35	9/15	60%
40	13/15	86.66%
45	13/15	86.66%
50	14/15	93.33%
55	13/15	86.66%
60	11/15	73.33%

Symptoms on pea seedling appeared within 10-12 day after transmission of virus showing leaf curling, leaf reduction, general stunting with or without yellowing. The incubation period of (PEMV) in pea aphid ranged between 4-6 hours.

1.3. Effect of inoculation access period (IAP) on transmission PEMV by pea aphid:

Inoculation access periods of (1-10 sec.), proved insufficient for successful inoculation of PEMV at 15 sec. proportion of infected plants was 6.66% and the highest proportion of infected plants was 100% Table 4. Symptoms on celery plants appeared within (7-9) days after their inoculation with the pathogen by the pea aphid. The symptoms on infected celery seedlings showing reduction in size of leaves crumpled and turn yellow along the edges and between veins.

1.4. Retention period of viruses in pea aphid:

The retention period pea enation mosaic virus (PEMV) by the pea aphid was 5 – 8 days.

From our results the pea aphid *Acyrthosiphon pisum* is an important natural vector for many plant viruses including Pea enation mosaic virus

Table (4): Effect of inoculation feeding period 0f aphid vector *Acyrthosiphon pisum* on transmission efficiency of Pea enation mosaic virus (PEMV) from infected pea plants celery plants.

Inoculation feeding period (Sec.)	Total number of healthy celery plants infected	Efficiency of virus transmission, %
1	00/15	00%
5	0015	00%
10	00/15	00%
15	1/15	6.66%
20	1/15	6.66%
25	3/15	20%
30	6/15	40%
35	6/15	40%
40	9/15	60%
45	10/15	66.66%
50	12/15	80%
55	14/15	93.33%
60	15/15	100%

(PEMV). Adult insects of A. *pisum* is able to acquire and transmit Pea enation mosaic virus (PEMV) .Also single insects are able to acquire (PEMV) and transmit it to pea plants. Minimum effective acquisition access and inoculation access periods are approximately 10-20 min. The rate of transmission increases with longer acquisition and inoculation access periods. A minimum of 4-6 hrs (latent period) from the time acquisition started is required for A. *pisum* to be able to infect pea test plants. In one insect/one plant inoculation test, nymph A. *pisum* are more efficient (95%) than adult female insects (25%). Viral DNA can be detected in single insects by PCR after 5 min of access feeding, and in pea plants as early as 5 min after inoculation feeding (Denis et *al*. 1982)

PEMV is a small, spherical virus which infects legumes. PEMV can be transmitted mechanically, through plant sap, however in nature it is transmitted in a persistent manner by at least 10 aphids of which pea aphid (A. *pisum)* and green peach aphid (M. *persicae*) are the most important. Minimum acquisition access period in nymphs is only 15 min, and in adults - 2 hours. PEMV has a temperature-dependent latency of 4-70 hours , followed by a minimum access inoculation period of 7-120 seconds. Because of this persistent mode of transmission, PEMV can be controlled through insecticide applications. Once acquired, the virus persists in the aphid body for the life of the insect and is retained through moulting, although it is not transmitted to progeny, and does not multiply in aphids.

Infected pea (*Pisum sativum* L.) plants develop a slight downward rolling of the trifoliate leaves 4-6 days post inoculation followed by a distinct yellow mosaic on the leaves. The yellow mosaic spots become translucent and clearly delineated. Later, plants develop dominance. Late in infection, diagnostic blisters or enations may develop on the underside of the leaves. Pods are often malformed and warty looking, and contain few if any seeds. The virus-vector relationship is in a circulative, non-propagative manner (Toros et *al*., 1978; Getz et *al*., 1982; Demler et *al*. ,1997,Timmermann-Vaughan et *al*. ,2009 and Dana & Millan, 2014).

The present study results of insects transmission experiments recorded the acquisition threshold feeding period ranged from infected pea plants to healthy celery1-20 min , from infected celery plant to healthy one 20-25 min ,and retransmission from infected celery plants to healthy celery plants 10- 20 min, inoculation threshold feeding period ranged from10-20 sec, incubation period in pea plants ranged from 8-11 days and in celery plants 7-9- days. Also, incubation period in A. pisum ranged 4-6hrs and the retention period of Pea enation mosaic virus (PEMV) in A. pisum was 4-5 days. This results obtained in this study agree with finding of some others (Bath & Chapman, 1966; Hull, 1981; de Zoeten and Skaf, 2001 and Hodeg &Powell, 2010).

They reported by a number of aphid species in a circulative persistent (non-propagative) manner. The virus can be acquired during access feeding periods of only a few minutes, and after a latent period the aphids can inoculate new plants in bouts of stylet probing less than 30 seconds duration and are disagree with other (Demler *et al*., 1996) due to the Daklia isolate of (PEMV) which may differ slightly in the biology and virus –vector relationship from those isolates described earlier, the number A. *pisum* used a similar conclusion was draw by El-Deffrawi *et al*.,(2000).

Moreover, the rate of transmission increased with increasing population density of the vector . The virus particles are subsequently transported through the gut wall into the hemocoel and from there they reach the salivary glands. The virus is Trans located into the salivary duct and is finally excreted with the saliva during feeding. The time it takes for a virus to complete this path is reflected in the minimal period of time that elapses from beginning of feeding on infected plants to transmission to test plants(latent period). This wide range latent period of values may reflect the efficiency with which a given virus establishes a systemic infection in a plant rather than differences in the velocity of translocation in the insect vector (Bath &Chapman, 1966, Tsai &Zitter,1982 and Sandy & reddy, 2020).

So these differences in PEMV transmission by aphids may be due to the potential vector aphid, the ,the proximity of the plants , environmental conditions, age of aphid, size of individual aphid strain of virus (Harris &Maramorosch1980,Abdel- wahab, 1998 and El-Deffrawi *et al*.,2000)

Conclusively, it can be concluded that transmission experiments proved that pea enation mosaic virus is transmitted by *A. pisum* in a persistent manner.

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كفاءه نقل فيروس مرض موزايك النموات الزائدة فى البسلة بواسطة حشرة من البسله بمحافظة الدقهلية فى مصر

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تعتبر حشرة من البسلة من الناقلات للعديد من المسببات المرضيه الفيروسيه بما فيها فيروس موزايك النموات الزائدة فى البسلة, وقد اثبتت التجارب التى اجريت على قدره حشرة من البسلة على اكتساب ونقل الفيروس من نباتات البسلة المصابه الى نباتات الكرفس السليمه ومن نباتات الكرفس المصابة الى نباتات الكرفس السليمه والى نباتات البسلة السليمة.

كانت اقل فتره تغذيه لازمه لاكتساب المسبب المرضى من نباتات بسلة مصابه تراوحت ما بين(1- 20 دقيقه)، وعلى نباتات الكرفس مصابه تراوحت ما بين

(15-25 دقيقه)، بينما كانت اقل مده لازمه لحقن المسبب المرضى تراوحت ما بين (15-25 ثانية).

كما اوضحت التجارب ان فتره حضانه الفيروس داخل حشرة من البسلة كانت مابين (4- 6 ساعه), وفى نباتات الكرفس ما بين(7- 9 يوم) وفى نباتات البسلة (8-11 يوم) وكانت قدره حشرة من البسلة على الاحتفاظ بالفيروس قد تراوحت ما بين(4- 5 يوم).

وقد اثبت التجارب على قدرة حشرة من البسله على نقل المسبب المرضى الفيروسى المسبب لموزايك النموات الزائده فى البسله.

التوصية:

من هذه النتائج تساهم فى فهم وبائيات انتقال الفيروس داخل مزارع البسلة فى مصر, كما ان العلاقه بين حشرة من البسلة ونقل الفيروس المسبب لمرض موزايك النموات الزائدة فى البسلة تسجل لاول مره فى مصر.