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Cotton Aphid (*Aphis gossypii* Glover) and Green Peach Aphid (*Myzus persicae* Sulzer) Efficiency of Zucchini Yellow Mosaic Virus Potyvirus (ZYMV) transmission on Squash Plants At Fayoum Governorate.

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ABSTRACT

Zucchini yellow mosaic virus (ZYMV) is an important non- persistent virus causing high squash production losses in Egypt. *Aphis gossypii* Glover and *Myzus persicae* Sulazer insects have high efficiency of ZYMV transmission from infected squash plants to healthy ones in Egypt,in advance of their rapid spreading. ZYMV was isolated from naturally infected squash plants collected from two locations (Sennoris & Fayoum), **at** Fayoum governorate. Virus identification was done by serological tests (ELISA) using four different antiserums. Virus was transmitted to healthy squash plants by mechanical transmission and by using the two aphid insects. Fecundity and life span of two aphids and forms (alate and apterous) were studied. ELISA test had relay that the virus was ZYMV. Typical symptoms of virus were observed by mechanical and aphid insect's inoculation, yellow mosaic, necrosis, leaf curling, blisters stunting, deformation, reduction in leaf size and knobbed fruits (sever malformation)**.** Fecundity and life span of *Aphis gossypii* were higher than those of *Myzus persicae* **on** infected squash plants compared with healthy, respectively.The reducing aphid insects population is recommended in order to decreasing losses caused by ZYMV infection (as their ability of transmission), in addition of removing the infected plants continuously which considered as a source if virus inoculum.

INTRODUCTION

Squash plants (*Cucurbita pepo* L.) are an economic crop in Egypt for local consumption. It can be produced almost as year round crop (Mohamed *et al.*, 2003). It is good source of minerals like iron, manganese, phosphorus, zinc and potassium in addition to its content of anti-oxidant vitamin-C and A (Whitaker and Bemis, 1976).

Viral diseases affect seriously the quality and quantity of agricultural products around the world, especially in less developed countries. Identification of viral plant diseases could be accomplished by several methods involving their morphological, physical, biological, cytological, serological and molecular properties (Naidu and Hughes, 2001; Lima *et al.*, 2012). Cucurbit crops were described to be infected by more than 20 viruses.

Zucchini yellow mosaic virus (ZYMV) is one of the most economically important viruses of cucurbit crops (Lecoq*et al.*, 1991; and Desbiez and Lecoq, 1997), which has a serious potential reduction of zucchini squash yield production, (Lecoq*et al*., 1991; and Shehata and El-Borollosy, 2008). It was firstly reported in California as being seed-borne in melons (Kendrick, 1934) and in Egypt at 1983 (Desbiez and Lecoq, 1997). ZYMV is a member of the genus *Potyvirus*, family *Potyviridae*, and exists as a long, flexuous virus particle, with a positive-sensess RNAgenome of750 nm long,and about 9600 nucleotides long with viral protein covalently 3- terminal, (Desbiez and Lecoq, 1997; Gal-On, 2007).The ZYMV-diseased cucurbit plants have systemic infection and causes mosaic symptoms in *Cucumis melo* L. (cantaloupe), *Cucumis sativus* L. (cucumber), and *Citrullus lanatus* (Thumb.) Mansfeld (watermelon) (Lisa *et al.*,1981; and Purcifull *et al.*,1984). Symptoms of zucchini yellow mosaic disease include sever mosaic, stunting, of the entire plant, yellowing , necrosis, leaf reduction, mottling and reduced flowering & distortion of existing flowers. The fruit is smaller, twisted, and often distorted by lumps, and it has a knobby appearance malformations (Lecoq *et al.*, 1991; Desbiez and Lecoq, 1997; and Kwon *et al*., 2005). It is transmitted by certain species of aphidsin anon-persistent manner, by plant sap containing the virus, and seeds borne (Lecoq *et al*., 2009).

The acquisition period of virus by aphids were rapidly during few minutes when moving or feeding on plant sap which infected by virus (Hunter and Ullman, 1992). It can be transmitted by 26 aphid species and mechanically (Perring *et al*., 1992; and Katis *et al*., 2006). There was two aphid species reported the highest transmission efficiencies' i.e., *Aphis gossypii* and *Myzus persicae*, being 35 and 41% respectively, (Castle *et al.*,1992).

Biological of aphid insects were affected by pathogen of these virus diseases. Barker(1960) found that, *Aphis fabae* Scopoli and *Myzus persicae* Sulzer were feeding of Beta plants which infected by Beta yellowing virus are more fertility and long of life span comparing by feed on healthy plants.

The present study aims to effect feeding range of two aphid species on squash plants infected by ZYMV on total progeny (fecundity) and life span of adult mothers comparing by another groups feeding on healthy plants; percentage rate of transmission by using the two aphid species for ZYMV and serological testes to identify different symptoms of ZYMV. All of these studies to make a good plan to control and prevent of spreading insect pathogen for getting highly squash production.

MATERIALS AND METHODES

Source of plant virus isolate

Young squash plants (*Eskandarani* cv.) were collected from two locations of Fayoum Governorate (Sennoris and Fayoum) districts, (50-plant samples/district) during summer growing season 2016. These samples were showing typical the symptoms of ZYMV, i.e., yellow mosaic, necrosis, leaf curling, blisters stunting, deformation, reduction in the size of leaf, knobbed fruits (sever malformation) & mixed pulp and sorted at 20^{o}C until use (Desbiez and Lecoq,1997; and Simmons *et al*.,2013).

Collecting aphids and identification

Insect aphid samples i.e., cotton aphid, *Aphis gossypii* Glover and green peach aphid, *Myzus persicae* Sulzer were collected from squashfields for rearing on healthy squash plant seedling (Eskandarani cv.) for two generations to take a pure culture of aphids (non-viruliferous individuals) and identified according to Blackman and Eastop(1984).

Experiment design

Squash seeds *Cucurbita pepo* cv. Eskandarani was sowed in 260 plastic pots (30cm diam., 25cm tall), and were divided (Fig. 1) as follow:

The first group (20 pots) was equally divided for each aphid specie for rearing in order to take a pure aphids culture. Only one adult virgin female from each species was put on healthy of 20-days old squash plants to be a source of infestation and insect transmission during the whole experimental period (Zehng *et al*., 2002).

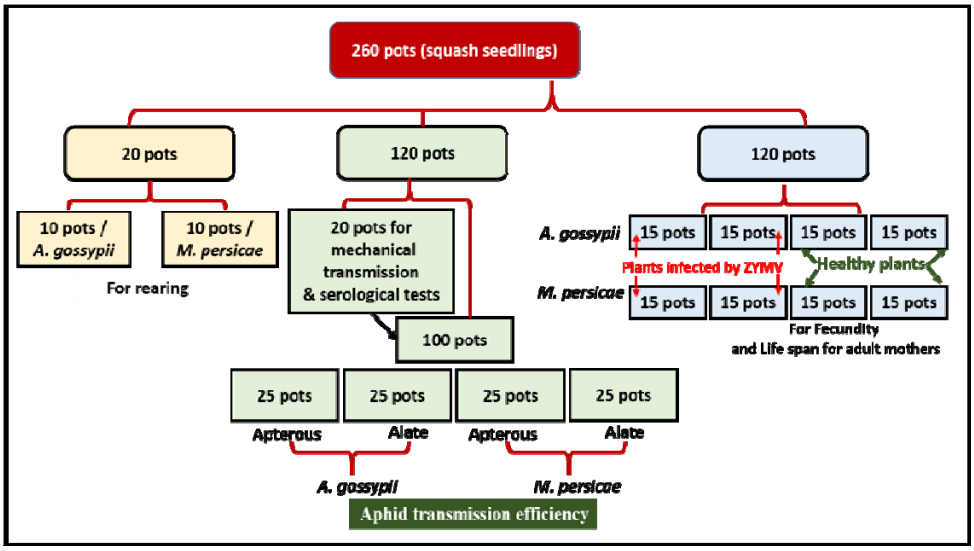

Fig. 1: The experiments design

The second group (120 pots) of healthy seedlings was divided into three sub-groups. The 1st sub-group containing twenty pots were used for mechanical transmission as shown in serological part. While, the 2nd sub-group containing hundred pots were used for inoculating by ZYMV from artificial infected squash plants to healthy plants to calculate the percentage rate of transmission for the two aphid species under studying. These pots were divided into four subgroups (25 pots/group). Each aphid species were introduced for each healthy squash plant after starved for one hour and placed for 30 minutes feeding (acquisition period) on artificial infected squash plants by ZYMV

The inoculation plants were monitored daily for 15-20 days until appearance of symptoms & comparing by healthy squash plants (control) and assayed by using Double Antibody Sandwich Enzyme Linked Immunosorbent (DAS-ELISA) by using anti-ZYMV polyclonal antibodies in department of virology in Plant Pathology Research Institute, PPRI, ARC, according to Clark and Adams (1977).

Finally, the third group (120 pots) of squash plant seedlings were divided equally into eight sub-groups. Each group contained (15 pots) for calculating fecundity, life span on both healthy & artificial infected squash plants for each aphid species. Numbers of progeny for each mother's aphid were recorded daily for each treatment and killed after counted till the end of mother's life, Aldryhim and Khalil (1995). Each pot was contained a mixture of soil and peat moss by rate of (2:1) and two seeds of squash. The seeds after germination, the plants were watered daily. Four granules per pot of complete fertilizer (nitrogen 12%; phosphorus 12%; potassium 17%; BASF-Asoco Agro, Germany) was applied in the growth medium once at seedling stage(20 days) (Soffan and Aldawood, 2014).

Virus identification

Mechanical virus inoculation:

Ten leaves from artificial infected squash plants by ZYMV were picked &blending in cold 0.1 M phosphate saline buffer pH 7.8 and diluted (1g: 1ml buffer w/v) . After that, was filtered through double layer of muslin and the filtrate solution was used as virus inoculums. twenty pots of seedling healthy squash plants were divided into two equal groups. The first group was

dusted with carborundum (600 mesh) at 3-4 leaf stage and inoculated with preparedinoculums' from infected leaves gently on the upper leaf surface of tested plants. Another second group seedlings were inoculated with buffer and served as a control. The inoculated plants were kept in insect-proof cages till symptoms development under labouratory conditions(monitored daily for 15-20 days).

Serological identification of virus:

Double Antibody Sandwich- Enzyme Linked Immunosorbent Assay (DAS-ELISA) was carried out to confirm and identify of the isolated virus at PPRI, ARC. The symptomatic squash leaf samples were tested using four different virus antiserums (Tab. 1). Crude sap extracted from healthy squash plant was served as control.

Table 1: Polyclonal antibodies used for virus identification

	Specific polyclonal antibodies for plant virus
1	Zucchini yellow mosaic Potyvirus(ZYMV)
2	Squash mosaiccomovirus(SqMV)
3	Cucumber mosaic cucumovirus(CMV)
4	Watermelonmosaic potyvirus(WMV)

Antisera were diluted to 1/100 using the coating buffer (0.05 M sodium carbonate, pH 9.6), then 100 µl were added to each well of the microtiter plate. The plate was covered and incubated at 37 ºC/3 hour. The plate was washed with Premier Biosoft Software International USA (PBST) buffer (136 M NaCl, 1.4 M KH_2PO_4, 7.9 M Na_2HPO_4, and 26.8 M KCl, pH 7.8). The buffer was left 3 min. in the wells. The washing was repeated 3 times, then the plate was emptied. The virus was diluted to 1/4 using sample buffer [PBST + 2% polyvinyl pyrrolidin(PVP)], then 100 µl of virus dilution were added to each well. The plate was covered and incubated overnight at 37 ºC. The plate was placed in the dark and examined after 30 min. using ELISA reader. the absorbans red at 405 nm and the reading were considered positive for infected plants but negative for control(Clark and Adams, 1977).

Aphid transmission efficiency:

The entomological study were conducted *in-vitro* at the department entomology faculty of agriculture, Fayoum university from the first of April till the end of September 2017at 25±3oC and 70 - 75% RH with a photoperiod of 16:8 L:Dh.

The two aphid species colonies and the two forms used for transmission experiment was initiated using a non-viruliferous single virginiparous female and reared healthy squash plants in an environmental growth chamber under controlled conditions as shown previously.

Groups of 25 individuals of each aphid species and forms were collected (Fig. 1), starved for one hour and placed, for 30 minutes (acquisition period) on ZYMV artificial infected squash plants which reared under room conditions and had been inoculated for one hour, then take it for two weeks to symptoms appearance. The two aphid species and forms (alate & apterous) which were fed on infected plants transferred to feed on healthy squash seedlings(one individuals form/aphid species/ one seedling) for one hour inoculation feeding period(Abd El-Wahab, 2012;and El-Borollosy, 2015), then killed. Assayed for two weeks later artificial infected plants were assayed for ZYMV infection using ELISA. Confirmation of aphid transmission of ZYMV to the healthy squash seedlings was conducted at the department of virology in Plant Pathology Research Institute, PPRI, ARC, according to Clark and Adams (1977).

Statistical analysis of data was analysed according to, Duncan(1955).

RESULTS AND DISCISSION

Isolation and Identification of virus isolate:

Isolation and propagation of virus isolate:

Zucchini yellow mosaic *Potyvirus* (ZYMV), was isolated from naturally

diseased squash (*Cucurbita pepo* cv. *eskandrani*) exhibited viral symptoms (Fig. 2), using mechanical and aphid transmission to healthy plants. After 2-4 weeks, inoculated plants developed symptoms similar to the original symptoms of ZYMV. These plants were kept inside laboratory conditions as source of virus (Fig. 3).

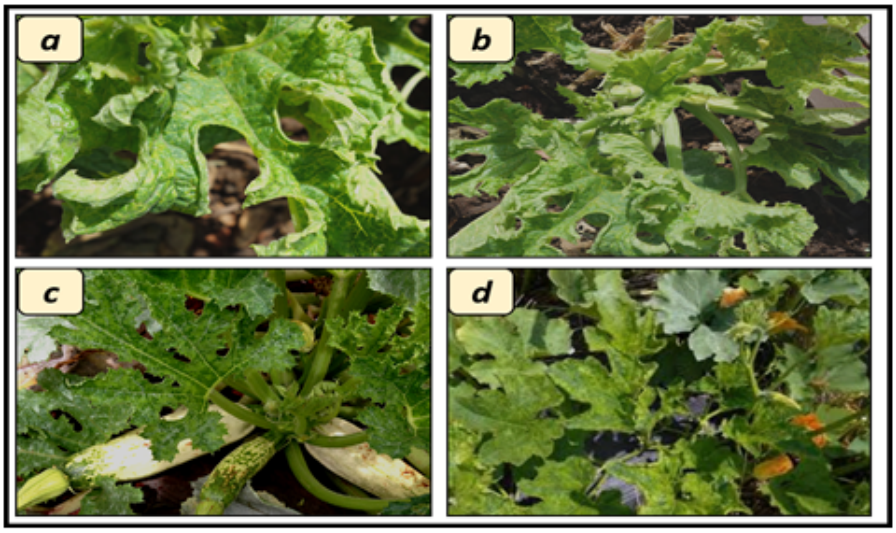

Fig. 2: Symptoms of ZYMV on squash plant leaves (a and b), flowers and fruits (*c* and *d*) natural infected under field condition at Fayoum governorate

Identification of virus:

ZYMV was identified according to their Symptomology and serological detection using direct ELISA Clark and Adams (1977).

Symptomology:

Field inspection of ZYMV viral disease was carried out firstly according to visual symptoms. Initial symptoms on squash including sever yellow, mosaic, blisters, reduction**.**

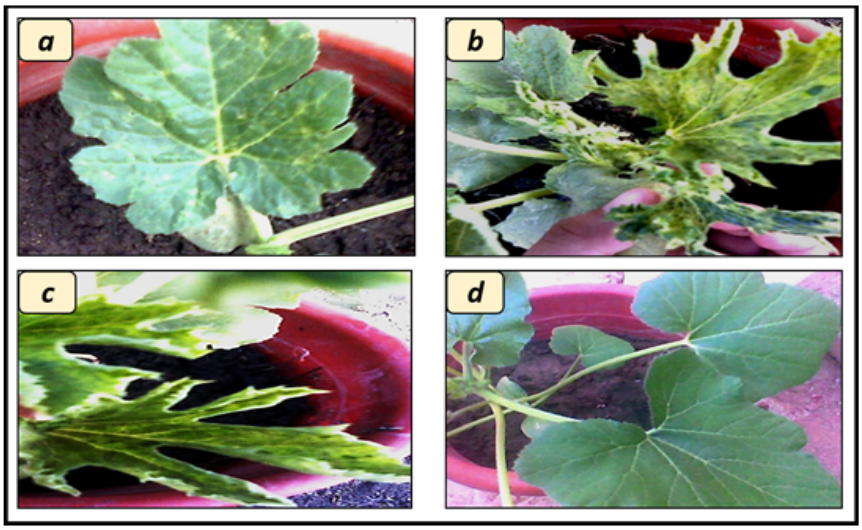

Fig. 3: Viral symptoms on Cucurbita pepo cv. Eskandrani plants were inoculated with the isolated virus, which is expected to be ZYMV; (a) mosaic and yellowing symptoms after 2-4 weeks from inoculation (b) sever blisters and reduction in size, (c) leaves cup upward and stunting (d) control plant under in-vitro.

Serological detection:

Naturally infected plants (*Cucumber pepo* cv. Eskandrani) exhibited viral symptom was tested by direct ELISA with four different viral specific antiserum. The data indicated that, all samples that were collected from the two districts at Fayoum governorate gave positive reaction with ZYMV. Zucchini Yellow Mosaic Virus (ZYMV) was isolated from squash plant showing like symptoms by depending on serology and symptoms (Shehata and El-Borollosy, 2008). The results obtained were in harmony with that found by Abdel-Reheem *et al*., (2006) who successfully detected ZYMV by Pc/Ic RT- PCR as a 1.3 Kbp helper components proteinase gene (Hc-pro) bands were detected within agarose gel, which had the same morphological symptoms.

Fecundity and life span for *A. gosspii* and *M. persicae*:

Five insects/each specie/each form/seedling plant were used for artificial infection (30 min. acquisition and 1 hr inoculation periods), in size. Leaves were infected by ZYMV, showed cup upward, developed intervene necrosis and stunting. Squash leaves may become distorted with regular marginal projections from the veins and fruits of infected plants. Fruits are also becoming malformation and mixed pulp, (Fig. 3).

Data presented in Tables (2) and (3) showed that, the mean numbers of *A. gosspii* fecundity (progeny numbers) were higher on artificial infected squash plants by ZYMV comparing by healthy plants being 118.1 and 55.8 nymph/apterous mo the respectively. While, *M. persicae* was 83.5 and 48.6 nymph/apterous mother respectively on infected and healthy squash plants, respectively.

On the other hand, the mean durations of life span (time length of apterous mother by days) for *A. gosspii* individuals were higher on artificial infected than healthy plants being 29.1 and 25.3days, respectively. The same trend was found for *M. persicae* individuals being 30.5 and 23.0 days, on infected and healthy squash plants, respectively.

These results are agreement with the finding by, Markkula and Lauren(1964) who found that, the reason of increasing progeny numbers (fecundity) and length of apterous virgin mothers (life span) for *A. gosspii* on natural infected squash plants by ZYMV than healthy plants due to highly sufficient of free amino acids concentration in infected plant virus than healthy plants. Highly concentration of these free amino acids in infected plant viruses by ZYMV were increased fertility of insect aphids. Also, Schubert and McRitchie (1984) reported that, infected squash plants should be rogued and destroyed whenever detected in field.

Table 2: The total numbers of progeny (fecundity) & life span by days for *Aphis gosspii* Glover on healthy and artificial infected squash plants by ZYMV under laboratory conditions.

Replicates	Plants infected by ZYMV		Healthy plants	
	Fecundity	Life span foradult mothers	Fecundity	Life span foradult mothers
1	145	33	36	17
2	82	20	70	25
3	85	25	44	27
4	127	31	86	33
5	147	33	59	29
6	157	36	73	35
7	77	27	50	23
8	128	32	31	25
9	100	22	39	22
10	101	25	65	29
11	126	26	63	35
12	131	34	33	22
13	174	33	85	26
14	80	24	74	15
15	111	36	29	17
Mean	118.1±7.11[b]	29.1±1.15[b]	55.8±5.36[a]	25.3±1.88[a]

Table 3: The total numbers of progeny (fecundity)& life span by days for *Myzus persicae* Sulzer artificial infected squash plants by ZYMV *in-vitro*.

Replicates	Plants infected by ZYMV		Healthy plants	
	Fecundity	Life span for adult mothers	Fecundity	Life span for adult mothers
1	66	25	34	12
2	75	30	51	15
3	56	29	42	20
4	84	35	33	17
5	99	28	47	19
6	101	27	59	29
7	87	26	37	24
8	65	29	50	18
9	77	31	49	22
10	105	38	35	23
11	93	27	61	30
12	74	36	38	28
13	90	24	55	27
14	88	37	70	29
15	93	35	68	31
Mean	83.5±4.23 [b]	30.5±1.40 [b]	48.6±4.71 [a]	23.0±1.32 [a]

Napier (2009) recorded that, there were numbers of aphid species attacked cucurbits including melon aphid, *Ahis gosspii*, cowpea aphid, *Aphis craccivora*, potato aphid, *Macrosiphum euphorbiae* and green peach aphid, *Myzus persicae*. Also, the obtained results and transmission efficiency were also in harmony with that performed by Varveri (2000).

Aphid transmission efficiency:

Data presented in Table (4) showed that, the highest efficient vector was found in apterous forms of *A. gosspii* and*M. persicae*, being 88 and 72% transmission rates, respectively. The lowest transmission rates were obtained in alate forms of *A. gosspii* and*M. persicae*, being 52 and 36% transmission rates, respectively.

Table 4: Percentage rate of transmission of ZYMV by using the two aphid species and forms.

Aphid species and forms		**Number of infected squash seedlings/25 plant tested by using artificial infection.**	
		Numbers	**%**
Myzuspersicae	Apterous	18/25	72
	Alate	9/25	36
Aphis gosspii	Apterous	22/25	88
	Alate	13/25	52

These results are agreement with the finding by, El-Borollosy (2015), reported that the winged forms of aphids were less efficient than the wingless ones in all tested species.

The most efficient vector for transmitting ZYMV was *M. persicae* and *A. gosspii* for the two forms. Where, transmission efficiency were (100 and 45%), and (85 and 25%) for wingless & winged forms, for the first and second aphid species, respectively. These results proved that the winged forms of aphids were less efficient than the wingless ones in all tested aphid species. Abd El-Wahab (2012) found that, the winged forms of sixteen aphid species were less efficient than wingless ones for transmitted Lettuce Mosaic Virus (LMV) in all tested species, in Egypt.

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ARABIC SUMMARY

كفائه حشرتي من القطن ومن الخوخ الأخضر في نقل فيروس تبرقش الكوسة الاصفر (ZYMV) لنباتات الكوسة بمحافظة الفيوم

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يعد فيروس تبرقش الكوسة الأصفر من أهم الفيروسات غير الباقية التي تصيب نباتات الكوسة وتسبب نقصا كبيرا في انتاجها بمصر. وتعتبر حشرتي من القطن ومن الخوخ من أكثر أنواع حشرات المن انتشارا بمصر وينقلا هذا الفيروس بكفائه عالية من نباتات الكوسة المصابة طبيعيا الي النباتات السليمة. لذلك تم عزل فيروس تبرقش الكوسة الأصفر من نباتات كوسة مصابة طبيعيا من مركزي سنورس والفيوم بمحافظة الفيوم وتم تعريفه سيرولوجياً، كما تم نقل الفيروس ميكانيكيا وحشريا وكذلك تم دراسة الكفائة التناسلية وطول فترة حياه الأم لنوعي المن تحت الدراسة وكذلك (المجنح منه وغير المجنح). أوضحت نتائج الدراسة تأكيد وتعريف الفيروس محل الدراسة سيرولوجياً ELISA باستخدام أربعة أمصال مضادة مختلفة. تم ملاحظة الأعراض المؤكدة للفيروس عن طريق النقل الميكانيكي والحشري من اصفرار وصغر حجم الأوراق وتقزم النباتات وتشوه الثمار. كذالك ازدياد اعداد النسل وزيادة طول فترة حياه الام لحشرة من القطن مقارنة بحشرة من الخوخ الأخضر علي النباتات المصابة مقارنة بالسليمة. كما ان نسبه نقل الفيروس للحشرات غيرالمجنحة كانت أعلي منه للحشرات المجنحة لحشرة من القطن يليها من الخوخ الأخضر. لذالك يجب الأهتمام بضرورة مكافحة حشرات المن لما تلعبة من دور قوي وفعال في نقل الفيروسات من النباتات المصابه للنباتات السليمة مع ضروره الأهتمام بازاله النباتات المصابه باستمرار لتقليل الضرر الناتج عن المسبب الفيروسي المنقول.