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The Detection of Homopterous Phytoplasma Vectors Infecting Some Ornamental and Medicinal Plants In Egypt

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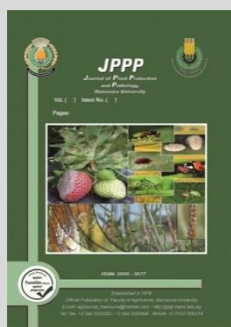


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ABSTRACT

This work is the first record of a phytoplasma associated with homopterous phytoplasma vectors infecting some ornamental and medicinal plants in Egypt. The field study was conducted during two successive seasons 2009 and 2011 in four different locations in Giza and Cairo governorates of Egypt, one was chosen and located inside Rose garden, Fac. of Agri., Cairo Univ., and the three others were collected from different fields located outside, in A-zohreiya gardens, Cairo governorate, Orman garden, Giza governorate and 6-October city. The infected sample of (rose, jasmine, rue, Egyptian henbane and periwinkle) plants were showing the symptom infection (phyllody, whitches-broom, little leaf and yellowing and revealed that the percentage of natural infection were (22.2, 82.7, 18.9 and 22.2%) while was 25% of Egyptian henbane virescence disease. Ten different identified species of field-collected insects (*Cicadulina bipunctella*, *Exitia taeniataiceps*, *Neolimniscus aegyptiacus*, *toya sp.*, *Empoasca sp.*; *Metatua sp.*; *Sogatella vibix*; *Chiasmus sp.*; *Balclutha sp.*; and *Chlorotettix sp.*) determined whether they can play a role in phytoplasma transmission or not. However, *Sogatella vibix*; *Chiasmus sp.*; *Balclutha sp.* and *Chlorotettix sp.* were not vectors of phytoplasma. With note, the presence of the psyllid (jumping plant-lice); infesting the ornamental/medicinal crops was detected, and that is the first record in Egypt

Keywords: rose, jasmine, rue, Egyptian henbane and periwinkle, phyllody, whitches-broom, little leaf, yellowing, virescence homopterous vectors, phytoplasma,



INTRODUCTION

In Egypt, the ornamental plants, Rose (*Rosa sp.*) (Rosaceae), and jasmine (*Gasminum gracilinum*), (Oleaceae), and the medicinal plants, rue (*Ruta graveolens*), (Rutaceae) and Egyptian henbane (*Hyoscyamus muticus L.*), (Solanaceae) are important plants and planted all over Egypt. Most of the native species of Family Rosaceae is sold annually worldwide as ornamental, and rose essential oil has high commercial value, (Sivaramane, *et al.*, 2008 and Madhupriya, *et al.*, 2017)), the flower of Jasmine is used as an aromatherapy, and the medicinal plant rue is used in traditional medicine as antispasmodic anthelmintic, and bacteriostatic, (Arias *et al.*, 2009). Egyptian henbane is a flowering plant grown in the desert arid areas of Egypt, eleven species are toxic and known as henbanes, while the main origin is an medicinal plant, and Egypt is producing and exporting for herb plants. Mikhail, *et al.*, 2012 report of phytoplasma affecting ornamental plants under Egyptian conditions and verified the presence of phytoplasma in tissues of diseased rose plants using electron microscopy and PCR based techniques.

Furthermore, phyllody, virescence, big bud, stolber and wiches-broom Phytoplasmas symptoms infecting of rose, jasmine, periwinkle and ornamental plants are associated with a wide range of ornamental plants worldwide and observed on rose, jasmine, rue and Egyptian henbane in Egypt, (Mohammed, 2013).

Phytoplasmas in nature are insect-transmitted bacterial pathogens, and they are phloem-limited, can

survive and multiply only in plant phloem or insect hemolymph, therefore, they are strictly host dependent. (Bertaccini *et al.*, 2014). Worldwide, only phloem-feeding insects can potentially acquire and transmit the phytoplasmas, most of them are classified in Order Hemiptera, mainly found among the series Auchenorrhyncha, (Elham, *et al.*, 2008), primarily insect vectors have been identified and confined to the leafhoppers (families Cicadellidae), four families of planthoppers (Fulgoroidea) and two genera of psyllids (Psyllidae), Amaral *et al.*, (2006), PNAS (2006). The potential of some "new" phytoplasmas to limit distribution of ornamentals are presented by Roger and Hei-Ti, (2006). More than 50 different ornamental plants species are infected by 11 different groups of phytoplasmas all around the world, and Phytoplasma diseases have been reported from many countries by (Madhupriya, *et al.*, 2017).

However, relatively few vectors species of phytoplasmas have been confirmed, (Weintraub, and Beanland, 2006 and Weintraub, 2007). Lee *et al.* 2000 (46) reported that around 200 vectors of phytoplasma are already known but many more are phytoplasma diseases characterized than are known vectors of diseases. So, there are many more described phytoplasmas than there are identified vector species. Molecular identification of phytoplasma (16Srl aster yellow group) disease, polymerase chain reaction (PCR) amplification of 16SrRNA (rDNA) sequence from various samples using pair P1 & P7, allowed the detection of the phytoplasmas into both ornamental and medicinal plants that showed

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symptoms, and in insects those have been trapped in any localities. (Mikhail, *et. Al.*, 2012 and Mohammed, 2013).

Forethemore, leafhopper species known to vector phytoplasmas never trapped before in any localities in Egypt except (Khalifa, *et.al.* 2008) reported the identification insect vector and molecular characterization of phytoplasma associated with the Al-wijam disease of date palms in Egypt.

Therefore, the present study aimed to verify the possible presence of phytoplasmas on some ornamental and medicinal plants serving as hosts for them in areas where the insects were collected, monitoring and identification of a putative vectors of phytoplasma disease those have been trapped in different locations in Egypt to determine their ability to transmit phytoplasma..

MATERIALS AND METHODS

This field study was conducted during two successive seasons 2009 and 2011 in four different locations in Giza and Cairo governorates of Egypt. One was chosen and located inside Rose garden, Fac. of Agri., Cairo Univ., and the three others were collected from different fields located outside, in A-zohreiya gardens, Cairo governorate, Orman garden, Giza governorate and 6-October city.

Assessment of natural infected plants

At all total location sites, 90 infected rose were taken, however 40 for each of the rest were taken. Samples of plants showing symptoms of possible phytoplasma infection, were labelled, weekly observed and categorized into groups according to the symptoms suspected to be caused by phytoplasma. Natural pathological symptoms of phytoplasma infection on plants were diagnosed through signs of phyllody, witches'-broom, yellowing, little leaf, and virescence. The percentage of disease incidence was calculated random samples according to the following equation:

$$\% \text{ of disease incidence} = \frac{\text{No. of infected plants}}{\text{Total no. of collected plants}} \times 100$$

All the infected plants were uprooted carefully in the greenhouse and were potted in 25cm pots filled with natural soil obtained from the experimental station of Fac. of Agric., Cairo Univ, (Mikhail, *et. al.*, 2012),to determine possible plant hosts.

Collecting leafhopper/planthopper specimens for identification and taxonomy by stereomicroscope.

All suspected insect vectors of phytoplasmas were captured and randomly collected weekly by net-sweeping and light trap at the Septembar 2010 - October 2011. The light trap has been conducted in the rose garden, and the sweep net sampling have been collected from all total location sites of (rose, jasmine, rue, and Egyptian henbane) plants where showing symptoms of phytoplasma infection had previously been found by (Mohammed, 2013). Collected adult leafhoppers and planthoppers were identified to the species level belonging to the Auchenorrhyncha families from graminaceous weeds as described by (khalifa, *et al.*, 2009) around chosen infected ornamental and medicinal plants by net-sweeping and trapping light.

All different specimens in total between leafhopper / planthopper species that caught were kept in plastic boxes and transferred to the laboratory and separated from each other as groups and each similar species in one group by

characters found in Elham *et. al.*, (2008). Specimens were prepared for microscopic examination and photographing by mounting on glass slide in a drope of Hoyer as found and used by Elham, (2008). They were slide – mounted and identified by stereomicroscope, in the Faculty of Agriculture Research Park, Cairo Univ., the digital images of the slide mounted were examined and photographed under a stereomicroscope LEICA DM750 and Fujifilm 5x digital camera. Identification of samples were made according to El-Nahal *et al.*, (1977 and 1979), Elbolok and Ammar (1986), Sewify and Herakly (1993), Elham, (2008) and Elham *et. al.*, (2008).

Sampling of viruliferous insects

The ability of field-collected species to act as vectors was tested to determine whether they can play a role in phytoplasma transmission or not according to (Fletcher *et al.* 1998), and kept under greenhouse conditions (22-25°C) as a source for the subsequent studies. Two groups of healthy plants were planted in pots and grown in an isolated greenhouse section, the first group was rose, jasmine, and periwinkle, as indicator host plants and the second was sorghum and barley plants for rearing the stock culture of insects. Otherwise, the similar field-collected species were divided into two groups, first, fed in groups (5-10 adults/plant) the second fed individually, on each indicator plant, via inoculation access period (IAP), and routinely weekly checked by allowing them to transfer and fed on new healthy indicator plants, then were determined the effect of infection on the presence of symptoms on indicator plants and the percentage of transmission by insect vectors were esimated. Indicator plants remained without presence of symptoms, thus proving that the feeding insects were non-viruliferous insects, and were transferred onto the second group of rearing plants as a stock culture, and were used as source of phytoplasma free insects.

The transmissibility of the phytoplasma and the percentage of vector transmission

Similar non-viruliferous species were removed from rearing culture and kept on infected naturally (sympatomatic) plants, for 7 days to acquire the pathogen, via acquisition feeding period (AFP). After (AFP), insects were transferred to healthy rose, jasmine and periwinkle seedlings individually 10-15 insects / 3replicates for an inoculation feeding period (IFP), for 2-3 weeks. The tested plants were kept in isolated section of greenhouse for 2-3 weeks following (IFP), and treated with insecticide to avoid any contamination, and observed for symptoms appearance to check the transmissibility of the phytoplasma. All transmission experiments were compared with untreated control indicators.

RESULTS AND DISCUSSION

The percentage of natural infection illustrated in Table (1), representing suspected to be caused by phytoplasma. Total of 210 samles of different ornamental and medicinal plants showing typical symptoms of phytoplasma diseases collected from different location in two (Cairo, Giza) Governorates. The infected sample of (rose, jasmine, rue, Egyptian henbane and periwinkle) plants showing the mentioned symptoms were tested by PCR and anatomical studies, Mikhail, *et. al.*, 2012 and Mohammed, 2013). Results reprected in Table (1) revealed that the percentage of mention symptom infection

(phyllody, whitches-broom, little leaf and yellowing) was recorded (22.2, 82.7, 18.9 and 22.2%) in (rose, jasmine and rue), respectively, while was 25% of Egyptian henbane virescence disease.

Table 1. Percentage of natural infection representing suspected to be caused by phytoplasma in two different governorates.

plant	Location /governorate	symptoms	No. sampl/infected plant	% inf.
Rose	Al-zohreiya/Cairo	Phyllody	90/20	22.2
	Rose garden/Giza	withches-broom	90/25	27.7
	Orman gar./Giza	yellowing	90/17	18.9
	6-october/Giza	little leaf	90/20	22.2
Jasmine	Al-zohreiya/Cairo	withches-broom	40/12	30
	Rose garden/Giza			
Rue	Orman gar./Giza	withches-broom	40/10	25
	6-october/Giza			
Egyptian henbane	Rose garden/Giza	virescence	40/10	25

Transmission of phytoplasma inoculation test by field collected species

Ten different identified species of field-collected insects (*Cicadulina bipunctella*, *Exitia taeniaceps*, *Neolimus aegyptiacus*, *toya sp.*, *Empoasca sp*; *Metatua sp*; *Sogatella vibix*; *Chiasmus sp*; *Balclutha sp*; and *Chlorotettix sp.*); either fed in groups (5-10 adults/plant); or individually, on indicator plants via inoculation access period (IAP) determined whether they can play a role in phytoplasma transmission or not. However, *Sogatella vibix*; *Chiasmus sp*; *Balclutha sp.* and *Chlorotettix sp.* were not vectors of phytoplasma. On the other hand, (Mohammed, 2013) provide and confirmed this result by PCR..

Acquisition and transmission phytoplasma by their vector species

The insects acquired the pathogen after keeping (7 days AFP) (7 days AFP) on naturally infected plants mentioned before, and transferred to healthy one, showed the ability in transmission of the phytoplasma to rose, jasmine and periwinkle plants. Whitches-broom and yellowing symptoms of rose, jasmine and periwinkle were observed on the tested plants after 21 days from infestation

the plants. The percentage of transmission by tested insects individually illustrated in Table (2)

Table 2. The transmissibility of the phytoplasma after (7 days AFP) by identified insect species

No. of replicates	Identificated insect species*						
	1	2.	3	4. Rose	5.	6.	7-10
1	08/10	05/10	10/10	10/10	08/10	06/10	0/10
2	09/10	08/10	10/10	07/10	06/10	09/10	0/10
3	06/10	10/10	08/10	09/10	06/10	04/10	0/10
Total	23/30	23/30	28/30	26/30	20/30	19/30	0/30
%	76.6%	76.6%	93.3	86.7	66.7	63.3	0%

No. of replicates	Identificated insect species*						
	1	2.	3	4. Jasmine	5.	6.	7-10
1	06/10	07/10	10/10	10/10	09/10	04/10	0/10
2	04/10	06/10	09/10	07/10	05/10	08/10	0/10
3	07/10	08/10	10/10	08/10	07/10	06/10	0/10
Total	17/30	20/30	29/30	25/30	21/30	18/30	0/30
%	56.7	66.7	96.7	83.3	70	60	0%

No. of replicates	Identificated insect species*						
	1	2.	3	4. Periwinkle	5.	6.	7-10
1	09/10	10/10	10/10	08/10	09/10	06/10	0/10
2	07/10	08/10	10/10	10/10	10/10	08/10	0/10
3	07/10	09/10	09/10	10/10	07/10	08/10	0/10
Total	23/30	27/30	29/30	28/30	26/30	20/30	0/30
%	76.6%	90%	96.7%	93.3%	86.7%	66.7%	0%

1-Metatua sp; 2-Empoasca sp; 3-Cicadulina bipunctella 4-Exitia taeniaceps 5-Neolimus aegyptiacus, 6-Toya sp. 7-Sogatella vibix 8-Chiasmus sp; 9-Balclutha sp 10-Chlorotettix sp.

Identification and abundase of the leafhoppers

Data arranged in Table (3) illustrated the population number of different leafhoppers/planthoppers species, (Fig.1); (*Cicadulina bipunctella*; *Exitianus taeniaceps*; *Neolimus aegyptiacus*; *Toya sp.*; *Metatua sp.*; *Sogatella vibix*; *Chiasmus sp*; *Balclutha sp*; *Chlorotettix sp* and *Empoasca sp.*). The following species have been caught in traps (2.872 adults) in relatively large numbers according: *Empoasca spp.* (840), *Chlorotettix sp.* (589), *Balclutha sp.* (583), *Toya sp* (580) and *Cicadulina bipunctella* (very low numbers 280), captured in Sweep Samples and light trap were identified from diferent surveyed fields during this study). The leafhopper, *Empoasca spp.* is trapped in relatively large numbers, (840 adults), while *C. bipunctella* has been trapped, although in smaller numbers, (280 adults). *Empoasca* leafhoppers, making up 29.2 % of the total leafhopper capture.

Table 3. Total population number of insects trapped by sweeping net and light traps collected from the different ornamental and medicinal fields

a- Family Cicadellidae,

a.1- leafhoppers genus *Balclutha*

Sep-010	Oct 010	Nov-010	Dec-010	Jan-011	Feb-011	Mar-011	Apr-011	May-011	Jun-011	Jul-011	Aug-011	Sep-011	Oct-011	Total
108	91	50	13	0	0	8	54	64	69	84	70	72	----	583

a.2- *Chlorotettix sp*

Sep-2010	Oct 2010	Nov-2010	Dec-2010	Jan-2011	Feb-2011	Mar-2011	Apr-2011	May-2011	Jun-2011	Jul-2011	Aug-2011	Sep-2011	Oct-2011	Total
64	63	29	8	0	1	13	55	89	95	90	50	55	32	589

a.3- Leafhoppers genus *Empoasca*

Sep-2010	Oct 2010	Nov-2010	Dec-2010	Jan-2011	Feb-2011	Mar-2011	Apr-2011	May-2011	Jun-2011	Jul-2011	Aug-2011	Sep-2011	Oct-2011	total
122	123	92	45	13	2	22	63	97	92	94	90	101	-----	840

a.4- *Cicadulina bipunctella* (Melichar)

Sep-2010	Oct 2010	Nov-2010	Dec-2010	Jan-2011	Feb-2011	Mar-2011	Apr-2011	May-2011	Jun-2011	Jul-2011	Aug-2011	Sep-2011	Oct-2011	total
21	28	20	0	0	2	10	24	50	28	44	27	26	21	280

planthoppers genus, *Toya sp.*

Sep-2010	Oct 2010	Nov-2010	Dec-2010	Jan-2011	Feb-2011	Mar-2011	Apr-2011	May-2011	Jun-2011	Jul-2011	Aug-2011	Sep-2011	Oct-2011	total
81	52	46	17	0	1	10	51	52	75	70	63	52	---	580

Total (2.872 adults)

a- Family Cicadellidae

leafhoppers genus *Balclutha* sp.

They are some of the most common leafhoppers in open grassland. Most species are coloured green although some of the *Balclutha* species are pink or red. They all feed on grass and some are important vectors of diseases of grass crops. Lui, *et al.*, 2013

***Balclutha rosea* (Scott)**

This species can be distinguished from *B. alstoni* and *B. saltuella* by the pair of hooked inner pygophore processes extending over the posterior margin laterally.

***Balclutha rubrinervis (viridinervis)* (Matsumura)**

This species is similar to the *B. punctata* complex and *B. cheesmanae* in external morphology, but can be distinguished by the pygophore with a posterior toothlike projection and aedeagus with the basal apodeme expanded strongly

***Chlorotettix* sp.**

Species in the genus *Chlorotettix* are usually green, or occasionally brownish. Most or all species have grasses as the host plants. Adult leafhoppers in this genus were made in the months between May and September, (Dwight, 1919).

Leafhoppers *Empoasca* spp.

Usually found on trees in summer and evergreens in winter, and adult all year. *Empoasca* species are pale green with pale markings, or are essentially unmarked on the head, pronotum and scutellum and to distinguish the genus from other leafhoppers, externally, *Empoasca* appears very similar to several other species, it can be reliably differentiated only by examination of the structures of the male genitalia.

***Cicadulina bipunctella* (Melichar)**

Cicadulina bipunctella (Melichar) has been trapped, although in smaller numbers (Table 2). There are two species of *Cicadulina* recorded that are very similar in external appearance, Kumashiro, and Matsukura, (2014).

b. Family: b. Delphacidae planthopper *Toya* sp.

Genus *Toya* according to the taxonomic keys of O'Brien and Wilson (1985) and Khalifa, E. (2008).

c. Psylloidea, (jumping plant-lice)

The presence of the psyllid (jumping plant-lice); infesting the ornamental/medicinal crops was detected (Fig.2), and that is the first record in Egypt. Psylloidea, or (jumping plant-lice), is a group of sternorrhynchous insects (Insecta, Hemiptera, Sternorrhyncha) which comprises 3000 – 3500 described species. Ossiannilsson 1992 suggested that the number of the actual existing species could be more than twice the number of the described species. Psyllids have a worldwide distribution, but they are most diverse in tropical and subtropical areas (Brown & Hodkinson 1988). There are six psyllid families (Calophyidae, Carsidaridae, Homotomidae, Phacopteronidae, Psyllidae, Triozidae), each family has a number of signature morphological characters (Hodkinson & White 1979). Psyllids have strong jumping legs and shorter antennae and that is what differentiates it from aphids (Ouvrard *et al.* 2002; Ouvrard *et al.*, 2008). Psyllids are known to closely resemble the Cicada insect, however adult psyllid species tend to differ in color (Adult psyllids are pale yellow or white with grey or black markings). Each kind of psyllid is fed “on only one plant species or closely related group of plants”(Ossiannilsson 1992). Adult psyllids look like psocids. The difference is in the fact that psocids have a more narrowed “neck” or there is a

separation between the head and the thorax in addition to chewing mouthparts. On the other hand, psyllids have tubular sucking mouthparts.

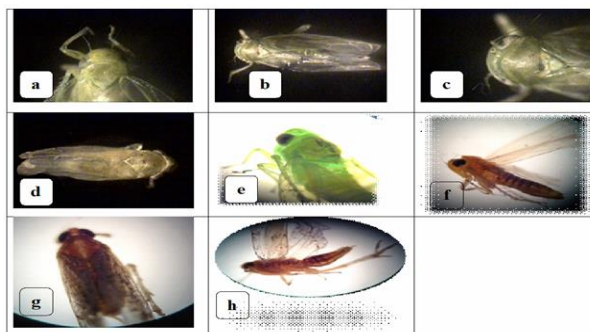


Fig.1. The different leafhoppers species, trapped by sweeping net and light traps collected from the different ornamental and medicinal fields; 1) Family Cicadellidae: a. *Balclutha rosea* (Scott); (b. and c.) *Balclutha viridinervis* (Matsumura); d. *Chlorotettix* sp.; e. *Empoasca* spp.; f. *Cicadulina bipunctella*(Melichar); 2) Family: Delphacidae planthopper, *Toya* sp.(g. female; h. male)

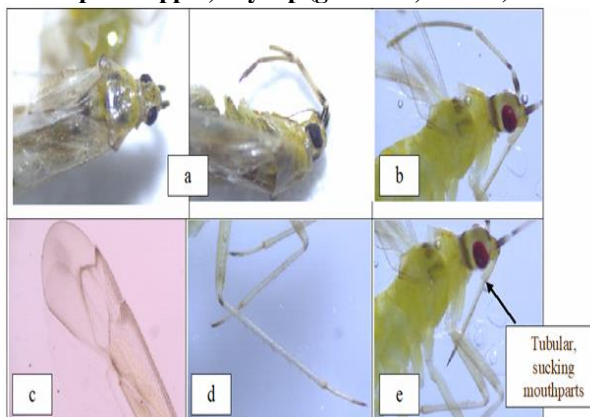


Fig. 2. The psyllid. a) The pale eyes with a dark spot. b) Shorter antennae than aphid and the tips of the antennae are grey. c) The transparent wings with yellow veins; Ouvrard *et al.* 2002 studied the comparative morphological assessment and phylogenetic significance of the wing base articulation in Psylloidea. d) Strong jumping legs. e) Tubular, sucking mouthparts as opposed to psocids' chewing mouthparts.

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الكشف عن نواقل الفيوتوبلازما متجانسة الأجنحة التي تصيب بعض نباتات الزينة والنباتات الطبية في مصر

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هذا العمل سجل لأول مرة في مصر النواقل الحشرية متجانسة الأجنحة المرتبطة بالفيوتوبلازما والتي تصيب بعض نباتات الزينة والنباتات الطبية. أجريت الدراسة الميدانية خلال موسمين متتاليين 2009 و 2011 في أربعة مواقع مختلفة بمحافظة الجيزة والقاهرة في مصر (ثلاث حدائق بمحافظة الجيزة حديقة الورد بكلية الزراعة - جامعة القاهرة ، حديقة الأورمان ومدينة 6 أكتوبر، وحديقة الزهرية بمحافظة القاهرة). أظهرت العينات المصابة طبيعياً من نباتات (الورد، الفل، والسداب، والسكران المصري) أعراض الإصابة (تشبه أعراض الفلودي - قصر عنق الأوراق وازدحامها على قمة النبات - مكنسة الساحرة - الاصفرار - اخضرار البتلات) وأظهرت نسبة الإصابة الطبيعية (22.2 ، 82.7 ، 18.9 و 22.2%) على التوالي، بينما كانت 25% لاختضار البتلات في السكران المصري. كما حددت عشرة أنواع مختلفة من الحشرات تم جمعها ميدانياً من المواقع المختلفة *Cicadulina bipunctata*, *Exitianus taeniaceps*, *Neolimnus aegyptiacus*, *toya* sp., *Emposca* sp; *Sogatella* sp; *Sogatella vibix*; *Chiasmus* sp; *Sogatella vibix*; *Chiasmus* sp; *Balclutha* sp; *and Chlorotettix* sp. وتم تقييمهم ما إذا كان بإمكانها أن تلعب دوراً كناقل للفيوتوبلازما النباتية أم لا. لتظهر *Balclutha* sp; *and Chlorotettix* sp. و *Balclutha* sp. لم يكونوا ناقلات للفيوتوبلازما. مع ملاحظة اكتشاف حشرة البسليد في حقول نباتات الزينة والنباتات الطبية أول تسجيل يحدث لها في مصر.