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## Efficacy of Certain Insecticides and Mineral Oil in Controlling Aphid, *Aphis gossypii* Glov. and Papaya Ringspot Virus in Squash at Kafr El-Sheikh Governorate

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### ABSTRACT

Melon aphid, *Aphis gossypii* Glov. is one of the most important insect pests infesting squash plants causing severe damage and is responsible for transmitting plant viruses. Among these viruses is, Papaya ringspot virus-W (PRSV-W) which, is a virus with the highest economic impact on cucurbits production. A two-year field experiment (2019 and 2020) was conducted at Kafr El-Sheikh Governorate to evaluate efficacy of three insecticides: Chess 50 % WG (pymetrozine), Tepeki 50 % WG (flonicamid) and Confidor 20 % (imidacloprid) as well as mineral oil (KZ 95 % EC) against aphids and spread of PRSV. The treatments were arranged in a randomised complete block design with three replications. During two seasons, tested compounds significantly reduced population of aphid vector, as well as the incidences of PRSV infections and increased fruit yield compared to control. Mineral oil had little effect on aphid populations but it was the best choice to reduce PRSV spread. Imidacloprid was the most effective insecticide against aphids but it was the least in reducing PRSV spread. Both antifeedant insecticides, flonicamid and pymetrozine significantly reduced populations of aphid and incidences of PRSV but they significantly increased fruit yield. Population of aphid was positively correlated with incidence of PRSV. Fruit yield was also negatively correlated with incidence of PRSV and aphid population. Overall, this work showed that it is possible to increase protection of cucurbits fields against PRSV and other non-persistent viruses by incorporating chemicals with different modes of action such as flonicamid, pymetrozine, and mineral oils.

**Keywords:** Mineral oil, *Aphis gossypii*, Papaya ringspot virus, squash fields, Kafr El-Sheikh Governorate.

### INTRODUCTION

Cucurbitaceae is one of the most important botanical families for human use as vegetable crops. In Egypt, squash, *Cucurbita pepo*, L. is considered one of the most popular vegetable crops, which are consumed in different ways as a food. In the fields, the squash plants are attacked by several insect pests. However, aphid insects, especially *Aphis gossypii* Glover, are one of the most important devastating pests infesting squash plants in the fields causing severe damage (Messing and Klungness, 2001). All over the world, aphids not only cause direct damage by sucking sap juices from the plants, exerting honeydew, which promotes the growth of sooty mold (*Capnodium* spp.) on leaves and harvestable plant parts, lowering their quality, but also act as viral vectors (Brault *et al.*, 2007)

Papaya ringspot (PRSV) is a serious viral disease of papaya and is considered one of the most important viruses which infects cucurbit vegetables worldwide, causing severe losses in the production (Mangrauthia *et al.*, 2008). Papaya plants of all ages are vulnerable to PRSV and generally exhibit symptoms after three to four weeks of infection. Many species of aphids transmit Papaya ringspot virus (Family: Potyviridae) in a non-persistent manner, among these, *Aphis gossypii*

Glov. is an efficient vector (Kallethwaraswamy and Kumar, 2008).

The transmission of non-persistent viruses is characterized by a short acquisition and inoculation access periods (seconds to minutes) as well as there is no latent period (Feres and Collar, 2001). Therefore, an ideal insecticide should kill aphids before they can accomplish the transmission process, which is rather difficult for non-persistent viruses taking also into account that many aphid vectors do not colonize the crop and they come in contact with the insecticide for a very limited period (Maelzer, 1986).

Applying mineral oil is a more effective way to prevent non-persistent viruses, as affects the infection process by preventing the development or translocation of viruses in plants (Peters and Lebbink, 1973) or physically interfere with the retention of the virus in the aphid's stylets (Boquel *et al.*, 2013). Another hypothesis is that the presence of mineral oil on or in the leaves can reduce the attractiveness of treated plants, thereby modifying the probing and feeding behavior of aphids (Ameline *et al.*, 2009, 2010).

Therefore, some chemical pesticides belonging to various chemical classes as well as mineral oil were studied against aphid, *Aphis gossypii* Glov., PRSV spread, and yield of squash.

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## MATERIAL AND METHODS

### Efficacy of certain insecticides and mineral oil against aphid *Aphis gossypii* Glov., and incidence of Papaya ringspot virus on squash plants:

#### 1- Plants and field experimental design:

The field experiment was conducted in the fall of 2019 and 2020 at a private farm in Kafr El-Shiekh governorate, Egypt. Seeds of squash, *Cucurbita pepo* L., cv. Eskandarani were obtained from Agricultural Research Center, Ministry of Agriculture, Giza, Egypt. The field was divided into 15 equal plots, each of 7.5m x 7.5m, and was separated by 2 m of bare soil on all sides. Each plot consisted of five rows. The plots were hand-seeded during the second week of September in both seasons with a plant spacing of approximately 70 cm between the plants. The missing plants were replaced after germination using squash transplants that were previously established in the greenhouse. Regular agricultural practices were applied according to the recommendations of the Ministry of Agriculture. A fungicide, azoxystrobin (Amistar,80%) was sprayed as required against powdery mildew during the early stages of the crop. Weed management was done mechanically as required.

#### 2- Virus source and inoculum plants:

The squash isolate of PRSV-W used in the experiment was initially isolated by Omar *et al.*, (2011) from Kafr El-Shiekh governorate, Egypt. To prepare plants for use as a PRSV inoculum source in the field. Squash was sown in plastic pots (11cm diam., 14cm high) and

maintained in controlled room conditions at 20±2 °C., 60±5% RH with 16:8h L:D photoperiod. Plants at the cotyledon stage were mechanically inoculated with the equivalent of 5 cm<sup>2</sup> of frozen stock tissue infected with PRSV-W (stored at -80 °C) and were used in the inoculation of the plants. Frozen tissue was ground, then combined with 15 mL of 0.1 M potassium phosphate buffer and carborundum powder, then applied to cotyledon surfaces using cotton swabs. All inoculations occurred from the same stock collection of frozen tissue. The inoculated plants were maintained in an insect-proof till the appearance of the virus symptoms. After that, squash plants were transported into the experimental plots (3 inoculated plants/plot) to serve as the PRSV-W inoculum source for all experiments as described below.

#### 3- Treatments:

Four treatments were evaluated using a randomized block design with three replicates in addition to a control check. The tested insecticides were Chess 50 % WG (pymetrozine), Tepeki 50 % (flonicamid), and Confidor 20 % (imidacloprid) in addition to one mineral oil (KZ oil 95 % EC). KZ oil treatment was conducted systematically every week, while each insecticide was applied four times; 21, 35, 49, and 63 days after sowing. Spray volume ranged from the equivalent of 60 liters/feddan at the cotyledon stage to 200 liters/feddan at the flowering stage. The leaves were sprayed to run-off using a high-pressure motorized 20 liters (Table,1).

**Table 1. The insecticides and mineral oil used in the study.**

Trade name	Common name	Chemical Class	Producer	IRAC Group	Dose
KZ oil (95% EC)	Mineral oil	Paraffin oil	Kafr El Zayat Pesticides and Chemicals	-	1 Liter/ 100 L water
Chess (50% WG)	Pymetrozine	Pyridine-azomethine	Syngenta Agro	9B	80 g/ Feddan
Tepeki (50% WG)	Flonicamid	Pyridine- carboxamide	SolfotecnicaSPA	29	20 g/ 100 L water
Confidor (20% SC)	Imidacloprid	neonicotinoid	Bayer Crop Science	4A	100 cc/ 100 L water

#### 4- Sampling:

##### 4.<sup>a</sup> Aphids.

A sample of ten leaves from ten plants was randomly selected from each plot. Aphids (adults and immatures) were visually counted in the field on the selected leaves in situ by the leaf-turn method as described by (Nyoike & Liburd, 2010). The sampling began 20 days after sowing (DAS) and continued every ten days till the final harvest.

##### 4.<sup>b</sup> Viral disease.

Visual observations of viral symptoms and incidence were monitored by recording the number of plants in each plot showing virus symptoms. The examination was done every ten days starting 20 days after sowing (DAS) and continued till 70 DAS. The infected plants were recorded. Symptoms of PRSV were initiated as vein cleaning, stunting, molting, mild or severe mosaic, malformed leaves, filiform, ringspot, and streak on fruits, stem, and petioles (Jain *et al.*, 1998). Plant samples (apical leaf) were further subjected to Standard Double Antibody Sandwich Assay (DAS-ELISA) LOEWE II Buffer for conjugate dilution for confirmation of PRSV presence.

The percentage of disease incidence was calculated by the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{No. of Infected Plants}}{\text{Total no. of Plants}} \times 100$$

Percentage of reduction in disease incidence over control was done as:

$$\text{Disease reduction (\%)} = \frac{C - T}{C} \times 100$$

Where: C is the percent disease incidence in untreated plants, T is the percent disease incidence in the treated plant.

##### 4.<sup>c</sup> Plant size sampling.

To estimate plant size, ten plants were randomly selected from the inner rows that had not been damaged during pest sampling. Plant size measurements were taken using a technique adopted by Frank & Liburd (2005). Plant height was measured from the ground to the terminal bud using a tape measure. The plant width was taken by measuring the length between the two widest opposing lateral shoots growing from the same plant.

##### 4.<sup>d</sup> Squash marketable yield.

Yield data were collected from the three inner rows of each plot that had not been damaged during sampling. Squash was harvested at the immature stage (soft, thin, edible rind shells) with edible seeds at approximately 20-25 cm long. Fruits were harvested and weighed in the field every other day for 3 weeks.

#### 5- Statistical analysis.

All the data were subjected to ANOVA and the significant difference among means of treatments were separated using Duncan multiple range test (Duncan, 1955) analysis using the CoStat software package, version 6.400.

## RESULTS AND DISCUSSION

### 1- Effect of certain insecticides and mineral oil against aphids on squash plants (*Cucurbita pepo* L.) under field conditions:

Efficacy of three insecticides (imidacloprid, pymetrozine, and flonicamid) in addition to mineral oil,

Table ) showed that all the treatments resulted in a significant reduction in the population compared to control. At 20 days after sowing (DAS), the aphid numbers were relatively low on treated and untreated plants ranging from 3.33 to 4.67 insects/leaf without a significant difference between the treated and the untreated plants (control). It was noted that the aphid population on untreated plants (control) increased steadily after 20 days of sowing (DAS) with a mean of 3.70 insects/leaf to 70 DAS with a mean of 10.5 insects/ leaf, while aphids on the treated plants with mineral oil and the tested insecticides showed fluctuation

KZ oil was evaluated against aphid population on squash, *Cucurbita pepo* L. plants under field conditions during two seasons; 2019 and 2020.

In the field observations, it was cleared that the major aphid species colonizing the squash plants were the melon aphid, *Aphis gossypii* Glov. In the first season, the results in and relatively lower population. At 30,40,50,60 and 70 DAS, the aphid population was higher on untreated plants than those on treated ones. Among the treatments, the mineral oil significantly was the least effective in reducing the aphid population, as the grand mean was 3.89 insects/ leaf (46.56 % reduction in population). The other treatments (insecticides) were highly effective against the aphid population with a mean ranging from 1.22 to 1.83 insects without significant difference between them. Also, the reduction in the aphid population varied from 74.86% to 83.24 % in the control.

**Table 2. Mean number of aphids on squash plants treated with mineral oil and insecticides during the season 2019.**

Treatment	Mean number/ leaf at indicated days after sowing							Reduction in population %
	20 days	30 days	40 days	50 days	60 days	70 days	Grand mean ± SE	
KZ oil	3.33	2.67	3.00	3.00	5.33	6.00	3.89 ± 0.34 b	46.56
Imidacloprid	4.00	1.00	0.33	0.00	0.67	1.33	1.22 ± 0.15 c	83.24
Pymetrozine	4.67	1.00	0.67	0.00	2.67	2.00	1.83 ± 0.42 c	74.86
Flonicamid	3.67	1.67	0.33	0.67	1.00	2.00	1.56 ± 0.15c	78.57
Control	3.70	5.63	6.85	7.29	9.65	10.5	7.28 ± 0.57a	----

In each column, means signed by the same letter are not significantly different at 5% according to Duncan's multiple range test (1955).

Regarding the second season (2020), the results were similar to those observed in the first season Table ). At 20 DAS, the aphid population did not significantly differ between the control and the treatments, as it ranged from 7.67 to 9.00 insects/leaf. After that, the population decreased in all treatments over time, while in control, the population gradually increased to reach 40.00 insects at the end of the season (70 DAS). Statistical analysis of data

exhibited a significant difference between the treatments as kz oil was the least effective against aphids recording a 52.79% reduction in the population. On the other hand, the insecticides induced the highest effect against aphids without a significant difference between them and the population reduction ranged from 84.50 (pymetrozine) to 86.33% (imidacloprid).

**Table 3. Mean number of aphids on squash plants treated with mineral oil and insecticides during the season 2020.**

Treatment	Mean number/ leaf at indicated days after sowing							Reduction in population %
	20 days	30 days	40 days	50 days	60 days	70 days	Grand mean ± SE	
KZ oil	8.67	6.33	7.00	8.67	15.67	21.67	11.33 ± 0.24 b	52.79
Imidacloprid	7.67	3.33	2.00	0.33	4.00	2.33	3.28 ± 0.55 c	86.33
Pymetrozine	9.00	4.67	0.67	0.00	3.67	4.33	3.72 ± 0.15 c	84.50
Flonicamid	8.00	5.33	4.03	0.82	1.48	1.87	3.59 ± 0.32 c	85.04
Control	8.00	12.00	17.33	31.67	35.00	40.00	24.00 ± 0.35a	----

In each column, means signed by the same letter are not significantly different at 5% according to Duncan's multiple range test (1955).

From the above-mentioned results, it can be observed that the aphid population differed from one season to other. Through the two seasons, the three tested insecticides significantly provided a high reduction in the aphid population, while the reverse was noted in the case of mineral oil. Also, field observations did not clear any phytotoxic effects on the plants. These findings emphasized the need to apply frequently oil sprays to achieve adequate aphid control.

The obtained results are in accordance with those of Mohamed & Homam, (2012), who reported that imidacloprid recorded the lowest population of *A. gossypii* on cucumber plants at Behira and Benisuef during the seasons of 2009 and 2010, while mineral oil gave an intermediate effect in reducing the population. Similarly, El-Dewy *et al.*, (2018) and El-Sherbeni *et al.*, (2018) found that imidacloprid and flonicamid recorded the highest reduction of aphid, *A. gossypii* on cotton plants.

### 2- Effect of certain insecticides and mineral oil on the incidence of papaya ringspot virus in squash plants under field conditions:

The results in Table ) indicated the efficacy of the different treatments in controlling the spread of papaya ringspot virus in squash under field conditions in the season of 2019. It was no observed virus symptoms at 20 and 30 days after sowing (DAS for all treatments, while a low incidence of the virus appeared on the untreated plants (control). After that, the infected plants increased over time, with the control being the highest in most cases. In the late season, the treatment with mineral oil significantly was the most effective recording a minimum incidence of papaya ringspot virus with a mean of 10.56 %, while the treatment of imidacloprid was the least effective in reducing the PRSV infection with a mean of 20%. The treatments of pymetrozine and flonicamid resulted in

relatively moderate effects with means of 13.33 and 14.44%, respectively.

**Table 4. Effect of certain insecticides and mineral oil on the incidence of papaya ringspot virus (PRSV) disease on squash plants during season of 2019.**

Treatment	% Incidence of PRSV at indicated days after sowing						Reduction in disease incidence over control (%)
	20 days	30 days	40 days	50 days	60 days	70 days	
KZ oil	0.00	0.00	1.67	4.44	8.89	10.56±1.06d	65.44
Imidacloprid	0.00	0.00	2.22	7.22	15.00	20.00 ± 0.69b	34.55
Pymetrozine	0.00	0.00	1.11	4.44	5.56	13.33 ± 0.81c	56.83
Fonicamid	0.00	0.00	0.56	2.78	6.67	14.44 ± 1.22 c	52.74
Control	0.56	6.67	8.33	15.56	20.56	30.56 ± 0.91a	-----

In each column, means signed by the same letters are not significantly different at 5% according to Duncan's multiple range test (1955).

With regard to the second season (2020), cleared in Table (5), the virus-infected plants increased over time, with the control being the highest. During the late season, it was observed that all the insecticides significantly recorded a lower incidence of PRSV as compared to untreated control (62.22 %) incidence at 70 DAS. The treatment with flonicamid was significantly superior over

the rest of the treatments showing 16.67% incidence, while imidacloprid was the least effective recording 41.67% virus incidence. The rest treatments; KZ oil and pymetrozine achieved a relatively moderate effect without a significant difference between them by 22.78 and 23.89 %, respectively.

**Table 5. Effect of certain insecticides and mineral oil on the incidence of papaya ringspot virus (PRSV) disease on squash plants during the season 2020**

Treatment	%Incidence of PRSV at indicated days after sowing						Reduction in disease incidence over control (%)
	20 days	30 days	40 days	50 days	60 days	70 days	
KZ oil	0.00	0.00	3.89	8.33	18.33	22.78 ± 0.92 c	63.38
Imidacloprid	1.67	1.67	2.22	11.67	25.00	41.67± 0.56 b	33.03
Pymetrozine	0.00	1.11	7.78	12.78	17.22	23.89 ± 0.98c	61.60
Fonicamid	0.00	0.00	3.33	10.00	13.89	16.67 ± 0.73d	73.21
Control	6.11	11.67	17.79	24.44	51.11	62.22 ± 0.87 a	-----

In each column, means signed by the same letters are not significantly different at 5% according to Duncan's multiple range test (1955).

The obtained results conformed with the finding of Pinese *et al.*, (1994), who reported that spray of mineral oils onto zucchini (*Cucurbita pepo* cv. 'Blackjack'), had some success in controlling the spread of stylet-borne (non-persistent) viruses, especially with frequent applications below phytotoxic levels. Similarly, Zitter & Ozaki, (1978) in Florida, USA, reported that mineral oil (Stylet-Oil) decreased virus spread by 3-8-fold in the experimental plots and offered complete protection under field conditions in squash, pepper, and cucumbers.

However, non-persistent viruses are more difficult to control by pesticide spraying because of their rapid acquisition and transmission properties that enable rapid spread from one plant to another (Radcliffe *et al.*, 2007). But insecticides can only reduce virus transmission if they affect the early stages of plant colonization, i.e. orientation, landing, and physical and chemical assessment of the plant before test probing (Perring *et al.*, 1999 and Broadbent, 1957).

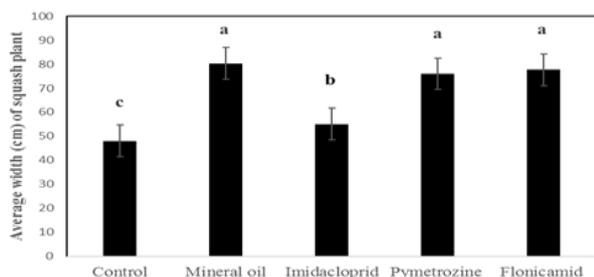
From the previous mentioned results, it was concluded that both pymetrozine and flonicamid were significantly better at providing a reduction in PRSV incidence compared with the control. Margaritopoulos *et al.* (2010) reported that pymetrozine suppressed transmission of non-persistent viruses by its antifeeding properties. Also, flonicamid has a strong feeding inhibition effect and kills aphids by starvation (Morita *et al.*, 2007). However, it seems that its antifeeding properties are similar to pymetrozine and by analogy, it may be useful in decreasing non-persistent virus spread in crops.

On the other hand, imidacloprid had no strong effect in inhibiting PRSV spread in squash. This agrees with the findings of (Alyokhin *et al.* 2002), who reported that imidacloprid might not be the most suitable mode of application for managing aphid populations and non-persistent viruses spread.

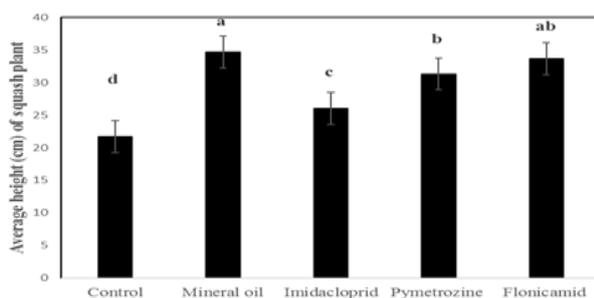
### 3- Effect of certain insecticides and mineral oil on plant size and fruit yield of squash plants:

The results in Fig. (1-6) reveal the effect of the three tested insecticides and mineral oil on the plant size (width and height) and fruit yield of squash during the two study seasons; 2019 and 2020. In the first season, it was noted that all treatments showed significant variations in plant size and fruit yield compared to the control (untreated plants). Also, among the treatments, mineral oil and flonicamid resulted in the highest plant height with means of 34.67 and 33.67 cm, respectively, followed closely by pymetrozine (31.33cm), while imidacloprid recorded the lowest plant height at 26.00 cm. Also, the same results were observed for the plant width. Similar results were observed in the second season.

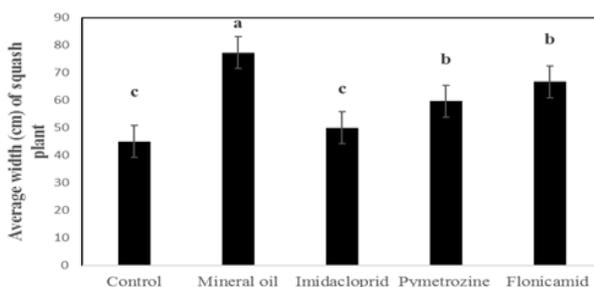
As for the fruit yield, plants treated with mineral oil significantly recorded the highest yield of 48.00 Kg /plot compared to the untreated plants (21.00 kg/plot), while the plants treated with imidacloprid gave the lowest yield of 28.00 kg /plot in the first season (Fig.5). During the second season, treatment of mineral oil, flonicamid, and pymetrozine resulted in the highest yield of 35.67, 32.00, and 30.33 kg /plot, respectively. Also, the lowest yield was achieved for the treatment of imidacloprid (22.33 kg) (Fig.6).



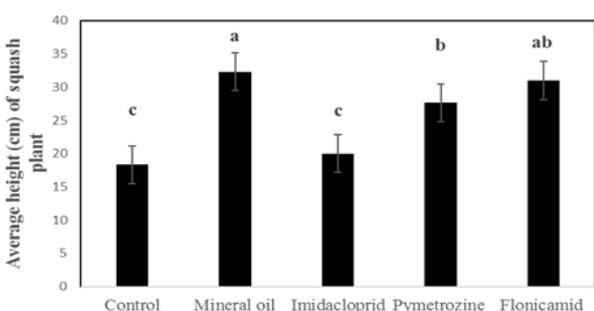
**Fig. 1. Effect of certain insecticides and mineral oil on width (cm) of squash plants during seasons 2019**



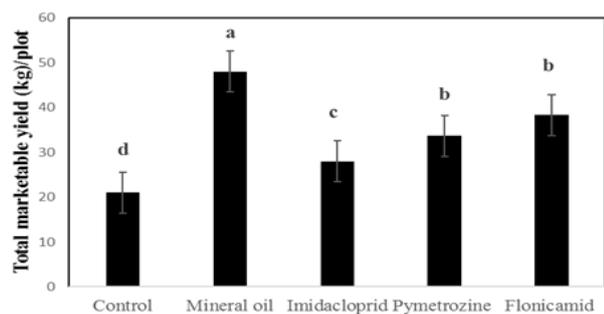
**Fig. 2. Effect of certain insecticides and mineral oil on height (cm) of squash plants during seasons 2019**



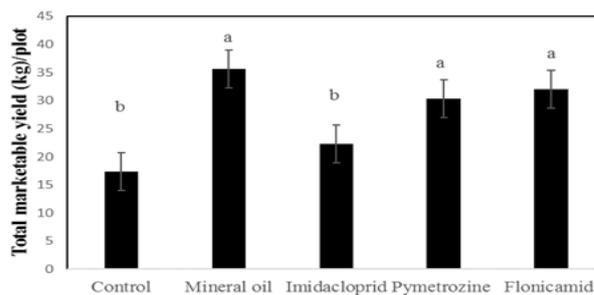
**Fig. 3. Effect of certain insecticides and mineral oil on width (cm) of squash plants during seasons 2020**



**Fig. 4. Effect of certain insecticides and mineral oil on height (cm) of squash plants during seasons 2020**



**Fig. 5. Effect of certain insecticides and mineral oil on fruit yield of squash during seasons 2019**



**Fig. 6. Effect of certain insecticides and mineral oil on fruit yield of squash during seasons 2020**

From the above-mentioned results, it can be noted that the high yield was correlated with the least virus infection and vice versa. Control treatment that had high virus infection recorded the least yield. On the other hand, the treatments that had high infection with virus-induced low yield as shown for Imidacloprid treatment.

**4- Relationship between aphid population, the incidence of PRSV, and fruit yield of squash:**

The results in Table (6) show the relationship between the aphid population, the incidence of PRS virus, and the fruit yield of squash during the 2019 and 2020 seasons. Statistical analysis indicated a positive and insignificant correlation between the aphid population and the incidence of PRSV virus in squash plants in the two seasons. But aphids exerted a negative and insignificant effect on the fruit yield. This may be due to the low number of aphid populations on the plants. On the other hand, the PRS virus had a significant effect on reducing the yield of squash.

**Table 6. Correlation coefficient between aphid population, percentage of incidence of PRS virus, and fruit yield of squash during 2019 and 2020 seasons**

	Season	Aphid population	Disease incidence	Fruit yield
Aphid population	2019	-----	0.677	-0.368
	2020	-----	0.753	-0.534
Disease incidence	2019	0.697	-----	-0.904*
	2020	0.753	-----	-0.941*
Fruit yield	2019	-0.368	-0.904*	-----
	2020	-0.5463	-0.941*	-----

\*= significant

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**فاعلية بعض المبيدات الحشرية والزيوت المعدنية في السيطرة على حشرة من البطيخ وفيروس التبغ الحلقي على نباتات الكوسا في محافظة كفر الشيخ، مصر**  
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تعتبر حشرة من البطيخ من أهم الآفات الحشرية التي تصيب نباتات الكوسا وتتسبب في أضرار جسيمة وهي مسؤولة عن نقل الفيروسات النباتية. من بين هذه الفيروسات، فيروس التبغ الحلقي في القرعيات، وهذا الفيروس له تأثير اقتصادي كبير على إنتاج القرعيات. تم إجراء تجربة حقلية لمدة عامين لتقييم فاعلية بعض المبيدات وواحد من الزيوت المعدنية على حشرة من البطيخ وانتشار فيروس التبغ الحلقي في حقول الكوسا. وكانت المعاملات المستخدمة هي مبيد تشيس (WG 50%) ومبيد تيببكي (WG 50%) ومبيد كونفيدور (SC 20%) والزيوت المعدنية هو زيت كزد (EC 95%) بالإضافة إلى المقارنة وتم تصميم التجربة في قطاعات كاملة العشوائية بثلاثة مكررات. خلال موسمي الدراسة نجحت جميع المركبات المستخدمة في تقليل أعداد حشرات المن ونسبة انتشار الفيروس مقارنة بالكنترول. الزيت

المعدني كان أقل المعاملات تأثيراً على تعداد حشرة المن ولكنه كان الأعلى تأثيراً في الحد من انتشار فيروس التبغ الحلقى . مبيد الايميداكلوبريد كان أعلى المركبات تأثيراً على تعداد حشرة المن ولكنه كان الأقل في التأثير على انتشار الفيروس. نجح كل من مبيدي الفلونيكاميد والبيمتروزين في التأثير بشكل معنوي على كل من تعداد المن وانتشار فيروس التبغ الحلقى مما أدى الى زيادة معنوية في المحصول. خلال موسمي الدراسة كان هناك ارتباط معنوي موجب بين تعداد حشرة المن وبين انتشار الفيروس وارتباط سالب بين المحصول وانتشار الفيروس وتعداد المن. بشكل عام، يُظهر هذا العمل أنه من الممكن زيادة حماية حقول القرعيات ضد فيروس التبغ الحلقى والفيروسات الأخرى غير الدائمة من خلال دمج مواد كيميائية ذات طرق عمل مختلفة مثل فلونيكاميد ، بيميتروزين ، والزيوت المعدنية.