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Effects of Certain Insecticides and KZ Oil on Survival of "*Aphis gossypii*" and its Transmissions Ability of Papaya-Ringspot-Virus "Prsv" on Squash

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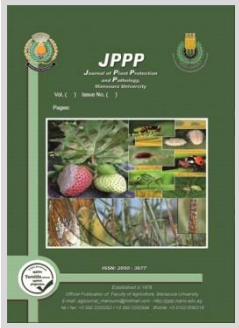

ABSTRACT

Aphids, particularly *Aphis gossypii* Glov., is one of the most-important-pests in squash farms, caused serious losses by sucking plant sap in addition to plant viruses' transmission such as Papaya ringspot virus-W (PRSV-W). The current work conducted to study of the effect of various-systemic-insecticides and mineral-oil against melon-aphid, "*Aphis gossypii*" and its ability on transmissions of non-persistent-viruses under laboratory conditions. Data obtained showed that the "flonicamid" and "pymetrozine" recorded significantly-reduction in virus-acquisition and inoculations when compared-with control "also" reductions were compared-with mineral-oil applications. On-other-hand, data obtained of insecticide-residues activity and KZ-oil on "PRSV" transmission-rate by "*A. gossypii"* revealed that flonicamid had significantly higher persistence up to 12 days followed by pymetrozine. The mineral oil experienced a significant increase in transmission rate as its efficacy decreased dramatically on the eighth transmission and twelfth days.

Keywords: KZ oil, survival**,** *Aphis gossypii*, Papaya ringspot virus, squash fields.

INTRODUCTION

In Egypt, squash, Cucurbita pepo, L., is one of most important popular-vegetable-crops which consumed in different ways. Also, the total production of cucurbits is 2,355,267 tons produced from area 84,512 hectares. Among cucurbits, squash produced total production of 406778 tons produced from 19750 hectares (data for 2019, FAOSTAT, 2020)

In squash fields, aphid insects especially *Aphis gossypii* Glover, considered serious-pests caused severely affect production of squash (Messing & Klungness, 2001). Around the world, aphids not only cause direct damage by sucking the plant sap, exerting honeydew, which support growth the sooty-mold "*Capnodium spp*." on plant-leaves and harvestable-plant-parts, and quality decreased, but also cause infection by transmitting viral diseases (Brault *et al*., 2007).

Papaya-ringspot-virus-W "PRSV-W" limiting-factor of cucurbit-production in the world-wide, Purcifull, 1984 and Mangrauthia *et al.* 2008. "PRSV-W" one of five-most-important-viruses that attacks vegetable-plants all over the world (Tomlinson, 1987). Its cause a several symptoms for cucurbit-crops such-as vein-clearing; stunting; mottling; mosaic; malformed-leaves; filiformy; ring-spots and fruits-streaks "stem and petioles" Jain *et al*., 1998. Also, its mechanically-transmitted and a non-persistent manners by at-least "25" different aphid-species (Purcifull 1984 and Kalleshwaraswamy & Kumar, 2008).

Application of effective insecticides against the virus vectors cannot always prevent spread of non-persistent-viruses (Simons & ZItter, 1980 and Lowery *et al*., 1990) because they do not work enough quickly against aphids before transferring virus infection to healthy-plants (Collar *et al*., 1997). Therefore, the control strategies of these viral diseases must be developed by using effective and environmentally safe measures in the integrated pest management programs.

However, mineral oils appeared to be effective in inhibition the spread of different non-persistent-viruses when frequently applied and at sub-phytotoxic-levels on potato-plants, Bradley *et al*.,1962, cucumbers, Loebenstein *et al*., 1964, peppers, Lange & Hammi, 1977, zucchini, Zitter, 1978, cucumbers, pepper and squash, Zitter & Ozaki, 1978 . Also, these oils decreased the aphids' ability to virus-attacks from infected-plants and transmitting to healthy-plants.

This study was carried out to determination of insecticides-effect and KZ-oil on "PRSV" acquisitions and inoculation by "*A. gossypii"* under laboratory conditions.

MATERIALS AND METHODS

Aphids

A clone of the cotton aphids, *Aphis gossypii* Glov. was collected from cotton plants cultivated at Kafr El-sheikh governorate, Egypt and maintained on virus-free squash plants in the Laboratory of Piercing-Sucking Insects Department, Sakha-Agricultural-Research-Station, Kafr-El-sheikh Gov., Egypt, at 23°C and 16 hrs light, 8 hrs dark.

Plant materials and cultivations

Squash-seeds were sown in seed trays containing perlite. The seedlings were grown under natural lighting (16:8 h dark/ night), temperature of around 25°C and a relative humidity of 60%. Seedlings that were one week old were planted in pots (2500 cm^3) using sterile soil mix (peat-vermiculite) containing 5g of slow release fertilizer, 20-20-20 N-P-K and trace micronutrients.

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Virus source and inoculum plants.

Squash plants at the cotyledon stage were inoculated using 1-2 young-leaves severely-symptomatic-tissue from infected-PRSV. Squash-plants were ground in 15ml. of 0.1m., potassium-phosphate buffer 7.0-pH. Plants at cotyledons were dusted with carborundum-powder to inoculate it.

The entire leaf surface was softly rubbed with sterile cheesecloth containing PRSV inoculum. To avoid excessive mechanical harm, the leaves were softly sprayed with water ten minutes after inoculation.

At two weeks after inoculation, newly-emerged-leaves of inoculated-plants displayed an intensive mosaic-pattern on lamina-leaf.

Insecticides and oil application

Three foliar insecticides and the mineral oil were applied to the squash pots (Table 1). A spray solution for each insecticide was prepared just before spraying immediately at recommended maximum-rate by manufacturer, Table 1. The tested mineral oil was a summer mineral oil. The summer mineral oil (KZ oil 95% EC) is paraffin-oil as a emulsifiable-concentrate formulation. In present study, after tests of preliminary effectiveness and phytotoxicity, KZ oil was applied at a dosage of 1%. Hand-held aerosol sprayers applied to insecticide and KZ oil to plant. Sprayers were kept 30cm away from plant during the application, and plants were sprayed to run-off. Each insecticide or oil was applied with its own hand-held aerosol sprayer.

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
Teppeki (50% WG)	Flonicamid	Pyridine- carboxamide	Solfotecnica SPA	29	20 g/ 100 L water
Confidor (20% SC)	Imidacloprid	neonicotinoid	Bayer Crop Science	4A	100 cc/ 100 L water

Experiment:

Effect of certain insecticides and KZ oil on *A. gossypii* under greenhouse conditions.

In this study, the methodology used was similar to that of (Martin *et al*., 2004). Squash plants in individual pots at (4-6) true leaf stages infested by 30 wingless aphid's adults (7-9 days old). Aphids were allowed to steady for about 30min. on plant. The tested-plants investigated for presence 30-aphids. Then, tested-plants sprayed uniformly, to run off by using small-hand-held sprayer. The three insecticides, mineral oil, and a water-treated control were sprayed on 10 aphid-infested-plants/treatments. After drying, plants were placed at a climate-chamber for about 16 hrs light and 8 hrs dark photoperiods and 22°C day and 16°C night. Aphid survival was determined on each plant at 16, 24, 48, and 72 hours after treatment.

Effect of insecticides and KZ oil on PRSV acquisition by "*A. gossypii*"

Experiments were conducted to studying aphids capability to virus acquires from sprayed-plants previously by using of different pesticides and mineral-oils. In this study, the methodology used was similar to that of (Martin *et al*., 2004), with some modification due to the different virus and vector studied. For each treatment, 7-young-leaves from single source-virus, chosen squash-plant were sprayed to the point of run off. Control plants were sprayed with water only.

After, one-hour drying-air, 30-aphids group putted/leaf and allowed to freely-probe for 5-minutes to virus acquire. Aphids then were transferred to 14-day-old healthy seedlings. Twenty plants per treatment (five aphids per plant) were allowed to probe freely for two hours for an inoculation the virus. After that, insects were removed manually from test-plants by hair-brush and then, tested plants transferred to aphids-proof-cages. After 3-weeks, the plants numbers appears characteristics "PRSV-infections" were estimated. The comparison of "PRSV-infection" between all-treatments and untreated control the chi-squared ($\chi 2$) test was used.

Insecticides effects and KZ-oil on PRSV-inoculation by "*A. gossypii*"

An assay was performed to studying "*A. gossypii*" capability to PRSV-transition to squash-plants that had been tested with different pesticides and oils. In this study, the methodology used was similar to that of (Martin *et al*., 2004), with some modification due to the different virus and vector studied. Twenty 14-day-old squash seedlings sprayed to point of run off for each treatment. Plants of control sprayed with tap-water. Following that, 25-aphids group starved for 2-hours, then putted on upper-leaves of source-virus on squash-plant and allowed to freely-probe 5-minutes. Then aphids were putted for sprayed on squash-plants 20plants/treatment (15 aphids/plant) and left to freely-probe 3-hours before removing by hair-brush from tested-plants. After that, the squash-plants transmitted to aphid-proof-cages. The tested-plant numbers after 30-days showed characteristics "PRSV-infection" was calculated. The comparisons of PRSV-transmission between all-treatments and untreated control, chi-squared $\chi 2$-test was used.

Residual-activity of tested-insecticides and KZ-oil on PRSV-transmission by "*A. gossypii*"

Based on the results of inoculation assay**,** the effect of insecticides (pymetrozine, flonicamid, and mineral oil) after the adding of viruliferous aphids at one of four different times was investigated in interfering with the transmission of PRSV at different days after a single application (residual activity) to squash transplants. Each of the tested compounds was sprayed on the plants grown in individual pots after one week of its emergence, while control plants sprayed with water alone. Seven to nine-day-old wingless aphids were starved for 2-hours and given 5-minute (AAP) on "PRSV-infected-plants, then transmitted to 20-healthy-plants 15-aphids for each-plant and left to freely-probe 3-hours. In each-treatment the tested-aphids removed manually from tested-plants by hair-brush (5 plants per replication; four replication). 1, 4, 8, and 12 days after treatment, the process was repeated. Experiments were repeated three times to ensure that the results were consistent. After 30 days, the infected-tested-plants proportion/treatment was noticing. Comparisons of PRSV-transmission between all-treatments and untreated control, chi-squared χ^2-test were used.

RESULTS AND DISCUSSION

Some insecticides and KZ-oil effect on *A. gossypii* under laboratory conditions:

Mortality rates of *A. gossypii* treated with the tested insecticides are summarized in Table 2.

At 16-hrs after aphid-introduction, mineral-oil caused low-mortality with mean of 18.33%, while imidacloprid significantly induced the highest mortality rate (95.67%). Pymetrozine and flonicamid caused 81.33 and 78.33 % mortality without significant differences between them. The efficacy of the treatments increased over the time as imidacloprid recorded 100% mortality after 48h. Pymetrozine and flonicamid caused 98.33 and 98.00%, while mineral oil was found to be the least effective with 34.67% mortality.

Table 2. Mortality rates of *Aphis gossypii* Glov. on squash plants previously treated with the mineral oil and insecticides.

Treatments	% Mortality± SE			
	16-hrs	24-hrs	48-hrs	72-hrs
KZ oil	18.33± 1.37 c	26.67 ± 0.79 d	31.33± 1.10 c	34.67 ± 1.14 c
Imidacloprid	95.67 ± 2.28 a	98.33 ± 2.10 a	100.00± 0.00 a	100.00 ± 0.00 a
Pymetrozine	81.33 ± 0.85 b	94.33 ± 2.15 b	97.67± 2.29 b	98.33 ± 2.10 b
Flonicamid	78.33 ± 0.79 b	91.33± 0.87 c	95.67± 2.63 b	98.00 ± 1.95 b
Control	____	____	____	____

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

Evaluation of insecticides and KZ oil against transmission of PRSV by *Aphis gossypii* Glover:

a- Effect of insecticides and KZ-oil on PRSV-acquisition by "*A. gossypii*"

In acquisition assay, the results in

Table) indicated that all treatments significantly reduced the transmission rate of PRSV compared to the control which recorded 60% (12/20) infected plants. The least infection rate was recorded in treatment of flonicamid, pymetrozine, and mineral oil with 5, 5.26 and 5.56 %, respectively as the reduction in transmission was 91.67, 91.23 and 90.74%. On the other hand, imidacloprid, was least effective in reducing PRSV transmission as it caused 50% reduction.

Table 3. Insecticides and KZ-oil effect on PRSV-acquisition *by "A. gossypii".*

Treatments	% Transmission rate	% Reduction in control	Chi-square χ^2 value	P-value
KZ oil	1/18 (5.56%)	90.74% **	12.4770	0.0004
Imidacloprid	6/20 (30.00%)	50.00% NS	3.6364	0.0565
Pymetrozine	1/19 (5.26%)	91.23% **	13.1368	0.0003
Flonicamid	1/20 (5.00%)	91.67% **	13.7892	0.0002
Control	12/20 (60.00%)	-----	----	---

Results are expressed as number of plants infected divided by the number of recipient plants

b- Insecticides and KZ-oil effect on PRSV-inoculation by "*A. gossypii*"

Results of infection rates obtained in inoculation test were shown in

Table). The highest infection rate of 70% (14/20) plants was recorded in the untreated plants. Flonicamid, pymetrozine and the mineral oil reduced the virus infection by 85.71, 77.49 and 78.57 %, respectively. Imidacloprid was less able to disrupt the transmission of PRSV by *A. gossypii* to squash plants, as the infection rate was 42.1 % (8/19).

Based on the previous findings, it can be stated that flonicamid, pymetrozine, and KZ oil were the most successful in limiting both PRSV acquisition and inoculation by *A. gossypii*. The reduction in infection rate was much higher in the acquisition assay than in the inoculation assay. This is in consistent with the finding of (Bradley *et al*., 1962 and Simons & ZItter, 1980), who found that oils had a more impact on acquisition than on inoculation. Imidacloprid was the least effective treatment, although having the highest mortality rate. In fact, some insecticides may promote virus transmission by stimulating aphid probing behaviour (Lowery *et al*., 1990 and Simons & Zitter, 1980).

The findings of this investigation are consistent with those of Martín *et al*., (2004), who reported that mineral oil completely inhibited cucumber mosaic virus (CMV) inoculation by *Myzus persicae*. Margaritopoulos *et al*. (2010) also found that one foliar treatment of pymetrozine offered adequate protection against potato virus Y (PVY) for 7 days.

Table 4. Insecticides and oils effect on PRSV-inoculation by "*A. Gossypii*".

Treatments	% Transmission rate	% Reduction in control	Chi-square χ^2 value	P-value
KZ oil	3/20 (15%)	78.57 **	12.3785	0.000434
Imidacloprid	8/19 (42.1%)	39.85 NS	3.0833	0.079098
Pymetrozine	3/19 (15.79%)	77.49 **	11.6451	0.000644
Flonicamid	2/20 (10%)	85.71 **	15.000	0.000108
Control	14/20 (70%)	----	----	----

Results are expressed as number of plants infected divided by the number of recipient plants

In addition, pymetrozine considerably reduced viral acquisition and inoculation by *Myzus persicae* compared to the untreated control and the reduction was comparable to a mineral oil treatment. According to Boquel *et al*., (2015), pymetrozine can reduce the spread of PVY by altering aphid behaviour. They also found that flonicamid reduced probing time for *M. euphorbiae* and *A. fabae* after application, lowering the possibility of effective PVY acquisition by the aphid and so contributing to the reduction of PVY spread.

The findings showed that the antifeeding and feeding cessation effects of some of the examined systemic insecticides are crucial in lowering the acquisition and inoculation rate of papaya ringspot virus (PRSV), which is transmitted by *Aphis gossypii* in a non-persistent manner. Therefore, both flonicamid and pymetrozine were found to be equally efficient in preventing PRSV infection and inoculation. As a result, these insecticides would be able to decrease, but not completely eliminate, PRSV's primary and secondary spread.

c- Residual-activity of insecticides and KZ-oil on PRSV-transmission by "*A. gossypii*".

Based on the results of inoculation assay, imidacloprid had low insignificant effect on reducing transmission rate of PRSV. Therefore, only the effect of pymetrozine, flonicamid and the KZ oil was estimated for residual activity on PRSV transmission by *A. gossypii*. The results in Fig. (1) showed that the residual effect of the two insecticides and mineral oil significantly differed, and the transmission increased in a stepwise manner as the days progressed. All treatments significantly reduced the transmission rate of PRSV up to four days after application compared to control. After that, transmission rate increased with the time. The high increase in transmission rate was observed for the mineral oil as, its efficacy reduced significantly on the 8th day (45% transmission) and the 12th day (70%). Pymetrozine and flonicamid provided significantly higher protection for up to 12 days. with 35 and 30 % transmission, respectively compared to the mineral oil

(70% transmission) and control (80% transmission). These findings point to the importance of frequent application of insecticides and oils to inhibit PRSV transmission.

The findings of the persistence study suggest that mineral oil should be applied on a regular basis to prevent PRSV transmission. The results of the current study are in conformity with those of Kalleshwaraswamy *et al*., (2012) who stated that the mineral protected papaya plants from papaya ringspot virus up to 4 days after application. On the contrary, Boquel *et al*., (2013) reported that the inhibition effect of mineral oil decreased with time after application; mineral oil inhibits PVY acquisition for less than 4 days at 5 l ha^{-1} .

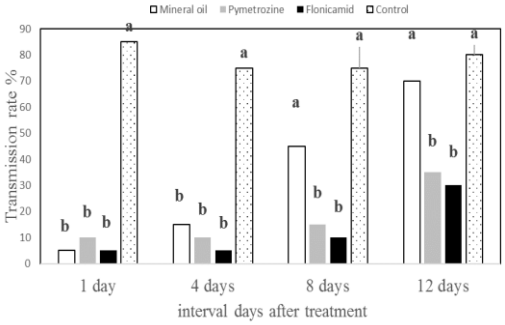

figure 1. Residual effect of single application of different treatments on the transmission rate of papaya ringspot virus (PRSV) on squash

[a] Results are expressed as number of plants infected divided by the number of recipient plants

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تأثير بعض المبيدات الحشرية والزيت المعدني KZ علي حشرة من البطيخ وعلي مقدرتها علي نقل فيروس التبقع الحلقي للباباظ علي نباتات الكوسة

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تعتبر حشرات المن وخاصة حشرة من البطيخ من أهم الحشرات التي تصيب حقول الكوسا والتي تسبب أضرارا بالغة وتؤثر بشدة على الإنتاجية كما أنه مسئول عن نقل الفيروسات النباتية مثل فيروس التبقع الحلقي للباباظ وكان الهدف من الدراسة تقييم فاعلية بعض المبيدات والزيوت المعدنية على حشرة من البطيخ الناقلة للفيروس وكذلك قدرة تلك المركبات على عملية التداخل مع عملية نقل الفيروس تحت الظروف المعملية. أوضحت النتائج أن كل مركبات فلونيكاميد وبيمتروزين والزيت المعدني قللت من قدرة حشرات المن على اكتساب وتلقيح الفيروس وبالتالي قللت من معدل نقل الفيروس بشكل معنوي مقارنة بالكنترول وفى تجربة الأثر الباقي أظهرت النتائج أن مبيد فلونيكاميد كان أعلى المركبات تأثيرا حيث وفر حماية نسبية للنباتات حتى ١٢ يوم بأقل معدل لنقل الفيروس تلاه مبيد البيمتروزين بينما ارتفع معدل الإصابة بشكل كبير في اليوم الثامن في النباتات المعاملة بالزيت المعدني.