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Measuring *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) responses to selected insecticides under greenhouse conditions

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Abstract

Bemisia tabaci (Gennadius) is a cosmopolitan insect pest of several crops worldwide. It has the ability of transmitting plant viruses, damaging, and developing resistance to pesticides. The aim of this work is to determine the efficacy of new chemical pesticides in comparison to current ones. Five selected insecticides, spiromesifen, pyriproquinazone, flupyradifurone, sulfoxaflor, and spirotetramat were used to control *Bemisia tabaci* on eggplants in greenhouse. Four consecutive sprays were carried at 10-day intervals. The efficacy was assessed by counting the living eggs and nymphs on plants before each spray. Results showed that pyriproquinazone was able to suppress the number of nymphs and the number eggs was very low 20, 30, and 40 days after the first treatment in comparison to all other treatments. The presence of a high population of whiteflies eggs and nymphs on the leaves sprayed with other insecticides indicates the development of resistance though not apparent with pyriproquinazone.

Keywords: Whiteflies, pyriproquinazone, pesticide resistance, tomatoes

Introduction

The sweet potato and cotton whiteflies, *Bemisia tabaci* (Gennadius) (Hemiptera, Aleyrodidae) is one of the most destructive insects to the agricultural sector worldwide. The economic importance of *B. tabaci* started in the late eighties principally in warm regions such as the tropical and subtropical ones [1, 4]. Moreover, the presence of *B. tabaci* has also been detected worldwide except Antarctica and it is considered to be one of the World's worst 100 invaders list. *B. tabaci* is a polyphagous piercing sucking insect that feeds on phloem sap of several plant species like vegetables (tomato, cucurbits, beans, cotton, potato, sunflower, and cassava) [5, 6], as well as many other crops including ornamentals causing direct and indirect damage to plants. Direct damage is characterized through phloem feeding leading to the reduction of the crop yield production by more than 50%. The annual losses due to the *B. tabaci* species in the "Middle East-Asia Minor 1" (MEAM1) are estimated to be US 714\$ million [7, 8]. Due to phloem feeding, whiteflies cause physiological damages and disorders known as "silver leaf", which are usually expressed in cucurbits crops and due to phytotoxins that are injected inside the leaf. As a result of phloem feeding, whiteflies secrete honeydew, which acts as a growth medium for sooty molds [5, 9, 10].

Indirect damage is associated with plants morphological malformation through vectoring a considerable number of viral plant pathogens that exceed 200 [11]. Plant viruses cause severe plant deformation and disorders. Diseased crops inflict significant losses in the agricultural production worldwide [1, 12]. *Bemisia tabaci* vectors and transmits mainly the most important plant pathogenic begomoviruses, of which the tomato yellow leaf curl virus (TYLCV) [10, 13]. In addition to viruses of the genus Begomovirus, *B. tabaci* transmits many other viruses belonging to other genera such as, *Carlavirus*, *Closterovirus*, *Ipomovirus*, and *Torravovirus* [14-17]. Recent research studies have reported that *B. tabaci* species complex consists of 39 reproductively compatible species with significant differences in biological characteristics but morphologically indistinguishable [18-21], including the widespread B biotype (MEAM1) and the Q biotype (MED) [22, 23]. Biological characteristics are related to the capacity of whiteflies species to build up a resistance to different insecticides [24, 25], the specificity to transmit viruses (*Begomovirus*) [11, 26], host-plant preference and range [27, 28], covering at least 1000 plant species [29], endosymbiotic characteristics, phytotoxic disorders [1, 30],

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the potential of invasion (invasiveness) ^[1, 2], and the reproductive incompatibility ^[6, 22, 31, 32].

Recently, two species of whiteflies showed a very high global invasive potential and an ability to substitute the native whiteflies species ^[2, 33]. These species are the “Middle East Asia Minor 1” (B biotype) and the “Mediterranean” (Q biotype), which are considered the most invasive²² spreading over 54 countries and over more than 10 countries for B and Q biotypes, respectively and associated with severe agricultural crop damage ^[34]. At the onset of the economic importance of the invasion of *B. tabaci*, farmers started to use chemical pesticides in order to control their population, the use of insecticides continues to be the basic approach and the commonly used method of control and suppression of whiteflies ^[35-37].

Whiteflies started to build up a rapid resistance evolution to commonly used and to an increasing number of insecticides over many countries worldwide leading to inefficient chemical control ^[37, 41]. Over the years, many insecticides have been used as a primary tool in the management of *B. tabaci* including chlorpyrifos (Organophosphate) ^[42, 43], neonicotinoids, such as imidacloprid, thiamethoxam, dinotefuran, and flupyradifurone ^[34, 36, 37, 44] and Sulfoxaflor ^[45, 46], the systemic anthralinic diamide, cyantraniliprole ^[46-50], insect growth regulators (IGRs), such as pyriproxyfen ^[25, 34], abamectine ^[34] and tetroneic acids, such as spirotetramat and spiromesifen ^[51, 52].

Despite the application of many insecticides, it is still very hard to control the population of whiteflies and thus reduce the transmission of viruses. The low efficacy of chemical control is due to the overuse or excessive application of insecticides classified under the same chemical group having the same mode of action. The misuse of insecticides such as the appropriate timing of spraying, the rates of use, and the susceptible stage of development of the whiteflies for an insecticide had led to rapid evolution and development of resistance to commonly used insecticides like Neonicotinoids and IGRs ^[53-55]. Many researches have shown that *B. tabaci* Q biotype shows high resistance to many insecticides in comparison to *B. tabaci* B biotype ^[56, 58].

The very low efficacy of some pesticides due to the fast buildup of resistance, pushed the farmers to introduce the resistance management approach as a new strategy for the control of *B. tabaci* under the integrated pest management based on the alternation and/or mixture of different insecticides with different mode of action ^[34, 35, 59]. In addition, the introduction of bio-control agents should be one of the major tools for the management of *B. tabaci* including parasites, predators, and entomopathogenic fungi like *Beauveria bassiana* ^[60].

The aim of this study was to determine the efficacy of the novel pesticides pyriproxyfen and flupyradifurone in comparison to spiromesifen, spirotetramat and sulfoxaflor against the whitefly *B. tabaci* on eggplant under greenhouse conditions. The results would be compared between the different pesticides efficacy on the eggs and nymphs developmental stages.

Materials and methods

Experimental set-up

The experiments were conducted on eggplant plantation (Lama Eggplant, Seminis) under greenhouse conditions in Aamchit, a coastal area of Mount Lebanon. The greenhouse surface is 132 m², 17 m long, 8 m wide, and 2 m high, divided

into 12 rectangular – shaped compartments.

Compartments were separated and built using a special insect proof net material in order to prevent any introduction of whiteflies, aphids and other insects. Each compartment was equipped by a door which is also made of insect proof net. The compartment walls were installed by fixing the net 40 cm below the soil surface and attaching the upper (opposite) side to an iron wire at 2m high.

The ceiling of all compartments is also made of special insect proof net that lays horizontally over the galvanized tubes of the internal greenhouse structure. Transplantation took place on May 24, 2018, plot doors were kept opened for 15 days in order to have a natural infestation of whiteflies on all seedlings.

Insecticides and their application

During the experiment, five insecticides were tested for their efficacy on whiteflies populations.

Pyriproxyfen (COLT 20WG, Nihon Nohyaku Co., LTD) (IRAC group 9B) (<http://www.irac-online.org>), primarily contact ^[64] and acts as translaminar insecticide suggested as a transient receptor potential vanilloid TRPV channel modulator ^[61, 62] for the control of sucking insects that belong to the families of Hemiptera and some Thysanoptera on vegetables, citrus and many other crops. Pyriproxyfen exhibits a fast feed cessation effect on treated insect ^[63].

Flupyradifurone (SIVNATO 200SL, Bayer crop science) (IRAC group 4D) (<http://www.irac-online.org>), a systemic insecticide ^[64] classified under the butenolide nicotinic acetylcholine receptor agonist group differing in its structure from the other known agonists ^[65] for the control of sucking insects on fruits, vegetables, and other crops ^[63].

Spiromesifen (OBERON 240SC, Bayer Crop Science) (IRAC group 23) (<http://www.irac-online.org>), inhibits the acetyl CoA carboxylase involved in the lipid biosynthesis process ^[66] leading to a sharp decrease of the total body lipid ^[67, 69]. Spiromesifen is a non-systemic insecticide affecting the development of whiteflies and mites by decreasing their fecundity. It also exhibits an ovicidal activity ^[63].

Spirotetramat (MOVENTO 100SC, Bayer Crop Science) (IRAC group 23) (<http://www.irac-online.org>), an ambimobile fully systemic insecticide ^[70], classified as lipid biosynthesis inhibitor affecting eggs and nymphs as well as adult fecundity of sucking insects like whiteflies, aphids and many others insects. It acts by contact and by ingestion. After leaf penetration, it exhibits a translaminar activity and it is translocated through xylem and phloem ^[63]. The effect of spirotetramat on immature stages is higher than that of adults of *B. tabaci* ^[51].

Sulfoxaflor (CLOSER 240SC, Dow Agro Sciences) (IRAC group 4C) (<http://www.irac-online.org>) is classified under the sulfoximines nicotinic acetylcholine receptor agonist group leading to a continuous excitation of the receptors and eventually to death ^[45, 71]. Sulfoxaflor controls the sap-feeding insects like whiteflies and aphids on vegetables, fruit trees, and many other crops ^[63].

The rates of use were calculated in function of the planted surface according to both the Lebanese and the US EPA registration sources. Spiromesifen 240 g L⁻¹, suspension concentrate (SC), was sprayed at an average rate of 500 mL ha⁻¹ according to the Lebanese registration and the approval of Bayer Crop Sciences (BCS). Flupyradifurone 200 g L⁻¹, water soluble liquid (SL) was sprayed at an average rate of 894.5 mL ha⁻¹ based on the US EPA registration.

Pyrifluquinazon 200g Kg⁻¹, water dispersible granules (WG) was sprayed at an average rate of 500 g ha⁻¹ based on Nihon Nohyaku registration (NNC - Japan). Sulfoxaflor 240 g L⁻¹, suspension concentrate (SC), was sprayed at an average rate of 325mL ha⁻¹ based on the Lebanese and US EPA registration. Spirotetramat 100 g L⁻¹, suspension concentrate (SC), was sprayed at an average rate of use of 750 mL ha⁻¹ based on the Lebanese ministry of agriculture registration and the approval of Bayer Crop Sciences (BCS). The rate of use for the different insecticides is shown in Table 1. The treatments were applied at 10 day-intervals as recommended by the companies, the first application was on June 8, 2018.

Table 1: Rate of use per square meter of the different treatments

Treatments	Rate of use	
	Formulated product mL/m ²	Active ingredient g/m ²
Spiromesifen	0.050	0.012
Flupyradifurone	0.089	0.018
Pyrifluquinazon	0.050	0.010
Sulfoxaflor	0.032	0.0078
Spirotetramat	0.075	0.0075

Data collection and analysis

Before each spray application, five leaves selected from the upper part of the plants, were collected randomly from each compartment, put in a plastic bag and transferred to the entomology laboratory at the Holy Spirit University of Kaslik. The last collection of leaves was performed 10 days after the last treatment on July 19, 2018. A total number of 50 leaves were analyzed before each sampling date and the total number of eggs and nymphs were recorded. The surface of each eggplant leaf is measured, and the number of eggs and nymphs was calculated as a function of the leaf surface. Numbers of dead and living eggs and or nymphs were recorded for each collection date. A total number of 250 leaves were analyzed during this experiment. For each treatment and at a particular date, the mean number of eggs and nymphs was calculated.

Table 2: Efficacy of insecticides applications on eggs of *Bemisia tabaci* per leaf at 10-day intervals.

Treatments	Number of eggs per leaf 10 days after each treatment ± SEs				
	Before treatment	10 days after first treatment	10 days after second treatment	10 days after third treatment	10 days after fourth treatment
Spiromesifen	883 ± 169	1646 ± 321 a	3480 ± 410 b	6942 ± 1472 b	11582 ± 2245 bc
Flupyradifurone	7058 ± 2308	8778 ± 1593 b	7663 ± 1241 b	10972 ± 959 b	23553 ± 3015 bc
Pyrifluquinazon	3946 ± 572	1487 ± 352 a	8 ± 4 a	121 ± 73 a	53 ± 17 a
Sulfoxaflor	3587 ± 663	15702 ± 2611 b	13363 ± 548 b	27571 ± 3117 b	25144 ± 3079 bc
Spirotetramat	6380 ± 1707	6027 ± 816 b	5461 ± 1607 b	18089 ± 1066 b	32694 ± 1402 c
Control	1970 ± 435	6683 ± 1309 b	15166 ± 1453 b	9520 ± 478 b	4014 ± 104 b

^a SEs: Standard error

^b All means in the same column followed by the same letter(s) are not significantly different at $P < 0.01$

Ten days after the fourth and last application or 40 days after the first application, Pyrifluquinazon was able to maintain a significantly lower number of eggs (53 ± 17) in comparison to all other treatments including the control ($F = 37.699$; $df = 5$; $P < 0.01$) (Table 2). From the other side, spirotetramat has recorded the highest number of eggs (32694); though, it was not statistically different from the number of eggs recorded in the treatments of Spiromesifen, Flupyradifurone and sulfoxaflor (Table 2).

Insecticides effects on nymphs

The nymphs of whiteflies were also best controlled by

For assessing the efficacy of the insecticides tested, the numbers of eggs and nymphs before the application were used as covariate and the values obtained 10 days after each treatment were analyzed through one way Anova; all values were subjected to Arcsine transformation in order to achieve homogeneity. The values were judged to be significantly different ($P < 0.05$) if their corresponding Tukey's 95% confidence limits did not overlap.

Results and discussion

To ensure that all compartments were relatively well infested with whiteflies, we proceeded to a first count before the application of the different treatments and the results of this first count indicated that all compartments were infested with *B. tabaci* (Tables 2 and 3). The sampling of the leaves was realized the same day of the first treatment.

Insecticides effect on eggs

Results have shown that there is a significant effect of treatment on the number of eggs of *B. tabaci* on the different dates post application. Ten days after the first treatment, the efficacy of the different insecticides was statistically different ($F = 10.473$; $df = 5$; $P < 0.01$). Both Spiromesifen and Pyrifluquinazon showed the least number of eggs and were significantly different than Flupyradifurone, Sulfoxaflor 240SC, and Spirotetramat, which were not significantly different from the control (Table 2). Following the second application by 10 days, Pyrifluquinazon has demonstrated a significant efficacy in comparison to all sprayed insecticides and recorded the least number of eggs/leaf (8 ± 4) in comparison to an infestation higher than 3000 eggs leaf⁻¹ for the other insecticides and the control ($F = 48.075$; $df = 5$; $P < 0.01$) (Table 2).

The third application took place also 10 days after the second spraying whereby Pyrifluquinazon has also showed a significant result in comparison to Flupyradifurone and all other insecticides in addition to the control and the number of eggs was 121 ± 73 ($F = 26.895$; $df = 5$; $P < 0.01$) (Table 2).

Pyrifluquinazon in all treatments (Table 3). Ten days after the first treatment both Spiromesifen and Pyrifluquinazon expressed a significantly higher efficacy in reducing the number of nymphs per leaf, 50 and 9 nymphs per leaf respectively, in comparison to Flupyradifurone, Sulfoxaflor, and Spirotetramat ($F = 10.377$; $df = 5$; $P < 0.01$) (Table 3). In addition, sulfoxaflor has recorded the highest number of nymphs of 1299 in comparison to all treatments (Table 3). Ten days after the second treatment, pyrifluquinazon and flupyradifurone has demonstrated a significant efficacy in comparison to spiromesifen, sulfoxaflor, and spirotetramat ($F = 6.693$; $df = 5$; $P < 0.01$). The lowest number of nymphs per

leaf was recorded in Pyrifluquinazon treatment and was 0, and the highest number was recorded in the spiromesifen treatment (Table 3). Following the third application by 10 days, Pyrifluquinazon maintained the number of nymphs equal to 0 in addition to Spirotetramat and were significantly different from the other insecticides in addition to the control ($F= 17.370$; $df= 5$; $P<0.01$) (Table 3). Forty days after the

first application and 10 days after the fourth application, Pyrifluquinazon revealed a significant efficiency and the number of nymphs was 0 ($F= 4.269$; $df= 5$; $P<0.01$). Spiromesifen and Spirotetramat were not significantly different from pyrifluquinazon and caused lower number of nymphs; however, they were not significantly different from Flupyradifurone, sulfoxaflor and the control (Table 3).

Table 3: Efficacy of insecticides applications on nymphs of *Bemisia tabaci* per leaf at 10 day-intervals.

Treatments	Number of nymphs per leaf 10 days after each treatment \pm SEs				
	Before treatment	10 days after first treatment	10 days after second treatment	10 days after third treatment	10 days after fourth treatment
Spiromesifen	371 \pm 104	50 \pm 35 a	452 \pm 225 ab	746 \pm 329 b	2152 \pm 1052 ab
Flupyradifurone	3248 \pm 949	769 \pm 185 bc	12 \pm 8 a	2093 \pm 733 b	2475 \pm 665 b
Pyrifluquinazon	4300 \pm 696	9 \pm 6 a	0 a	0 a	0 a
Sulfoxaflor	6453 \pm 1059	1299 \pm 644 abc	225 \pm 70 ab	2568 \pm 974 b	2052 \pm 540 b
Spirotetramat	9785 \pm 1646	208 \pm 144 ab	89 \pm 47 a	0 a	2098 \pm 1182 ab
Control	1858 \pm 499	9819 \pm 1437 c	7950 \pm 1532 b	9712 \pm 492 b	3446 \pm 1010 b

^a SEs: Standard error

^b All means in the same column followed by the same letter(s) are not significantly different at $P<0.01$.

Pyrifluquinazon showed the most efficacious results in comparison to all other used insecticides in the experiment. The high efficacy of pyrifluquinazon was revealed by the very low number of both eggs and nymphs on eggplant leaves. The statistically significant low number of eggs is mainly due to the high mortality of adults of *B. tabaci* treated with pyrifluquinazon. The high efficiency on eggs was elucidated in the significant decrease from 3946 ± 572 (Table 2) before any treatment to 1487 ± 352 , ten days after the first treatment, then to 8 ± 4 , ten days after the second treatment, then to 121 ± 73 after the third treatment, and to 53 ± 17 ten days after the fourth treatment (Table 2).

On the other side, the number of nymphs has also decreased significantly from 4300 ± 696 before the first treatment to 9 ± 6 , 0 , 0 , and 0 nymphs ten days after the first, second, third, and fourth treatments, respectively (Table 3). This sharp decrease in the number of nymphs within ten days could be an indication that some of the insects were controlled as emerged adults, another portion was controlled as nymph and the remaining population was directly controlled after egg hatch as neonates or crawlers as obtained by Smith and Giurcanu [64], taking into consideration that pyrifluquinazon is not an ovicide [63]. The effect of pyrifluquinazon (pyridine azomethine derivative insecticide) on sap sucking insects is characterized by the modification (perturbation) of the coordination and the insect feeding behavior leading to feed cessation followed by death [61, 72].

In the treatment of flupyradifurone, the novel insecticide, the numbers of eggs and nymphs were higher in comparison to pyrifluquinazon. The number of eggs was not statistically different from the untreated plot. As shown in Table 2, and starting ten days after the second application, the number of eggs increased significantly from 7663 ± 1241 to 10972 ± 959 and just before the fourth application the number increased to 23553 ± 3015 , ten days after the fourth and last application. The sharp differences are due to the low efficacy of flupyradifurone against the adult whiteflies as well as on their fecundity and fertility. The effect of flupyradifurone on nymphs fluctuated as it showed high efficacy 10 days after the second spray (12 ± 8) and then it decreased ten days after the third and fourth treatment, 2093 ± 733 and 2475 ± 665 , respectively, demonstrating that its efficacy on larval stage is not consistent.

Sulfoxaflor, the second active ingredient of nicotinic

acetylcholine receptor (nAChR) competitive modulators group (IRAC group 4C) (<http://www.irac-online.org>) showed insignificant differences in comparison to the untreated plot indicating insufficient control potential of both eggs and nymphs (Tables 2 and 3).

The low efficacy of sulfoxaflor and flupyradifurone may be attributed to the building up of metabolic cross resistance since they are classified under the same group (nAChR) IRAC group, 4C and 4D, respectively (<http://www.irac-online.org>), knowing that the resistance development to the neonicotinoids has been already demonstrated [44]. This hypothesis is not in conformity with some studies mentioning the absence of metabolic cross resistance in *B. tabaci* to flupyradifurone since the overexpression of a specific gene (CYP6CM1) is responsible of the hydrolysis of imidacloprid and pymetrozine in *B. tabaci* and not flupyradifurone. In other studies, no metabolic cross resistance to flupyradifurone has been revealed [65]; however, Smith *et al.* [37] mentioned that flupyradifurone revealed some cross resistance to neonicotinoids in Florida. Other studies have also demonstrated that sulfoxaflor has no cross resistance with other neonicotinoids [46, 73], and some researches demonstrated a field resistance evolution to sulfoxaflor and thiamethoxam [74].

Spiromesifen and spirotetramat belong to the group of tetrone and tetramic derivatives and are more effective on immature stages than on eggs (Tables 2 and 3). This hypothesis has been confirmed by other works [67, 75]. Spiromesifen demonstrated significant efficiency on eggs (1646 ± 321) only after the first spraying where the number of eggs was relatively low (Table 2). While for all other treatments and dates of spraying, including the first treatment of spirotetramat, the numbers of eggs were not statistically different from the untreated control plot. Chen *et al.* [74] obtained similar results with respect to the low efficacy of spirotetramat on eggs. In general, tetrone acids demonstrated moderate efficiency on nymphs especially spirotetramat which demonstrated a higher efficacy on nymphs in comparison to spiromesifen (Table 3). The moderate efficacy indicates certain degree of resistance [74, 75]. The efficacy of spirotetramat is in concordance with the results obtained by Peng *et al.* [75] which revealed a significant effectiveness on nymphs and not on eggs.

It should be pointed out that the number of both eggs and

nymphs in the untreated plot has decreased during the experimental period. The number of eggs has decreased from 15166 ± 2453 to 9520 ± 478 , and then to 4014 ± 104 , ten days after the second third and fourth treatments, respectively (Table 2). Nymphs' populations also decreased from 9712 ± 492 to 3446 ± 1010 (Table 3) ten days after the third and fourth treatments respectively. The high population of eggs, nymphs and adults during the first half of the experimental period has led to a significant shortage of the plant growth eventually followed by a sharp decrease of *B. tabaci* population (Tables 2 & 3).

The highest and significant efficacy was demonstrated for Pyriproxyfen in comparison to all used insecticides in the experiment. *B. tabaci* hasn't showed any degree of resistance development to pyriproxyfen; in addition, pyriproxyfen showed an excellent control of first instar nymphs and adults at very high population. Thus, pyriproxyfen should be recommended as an important tool in the management of *B. tabaci*. Regardless of the use of a new molecule flupyradifurone, it was clear from our study that active ingredients of nicotinic acetyl cholinesterase receptor inhibitor (competitive modulators) were always prone to whiteflies resistance/cross resistance at all levels of the life cycle. Despite of the beginning of the emergence of resistance, tetracycline acids showed moderate efficacy in comparison to pyriproxyfen and sometimes significant differences at the level of eggs and nymphs in comparison to (nAChR competitive modulators) group.

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