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Rapid Bioassay for Detection of Insecticide Resistance in *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae)

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ABSTRACT
Cotton aphid, *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) is one of the most economically important polyphagous pests that shows rapid resistance to chemical control. Determination of resistance levels is important within resistance management. The insecticide activities of Acetamiprid, Dimethoate, Imidacloprid, L-cyalothrin were investigated on different aphid populations to develop a faster and more economical bioassay method. Resistance levels were compared by making bioassays with insecticides using the leaf dipping method for 72 hours, 120 minutes and different doses. Resistance rates varying between 1-10 times were observed in both methods. The rapid application of 120 minutes is more advantageous in terms of speed, application time and economy of the method in the detection of resistance against Acetamiprid, Dimethoate, Imidacloprid, L-cyalothrin insecticides, and will contribute to the detection of resistance and the development of bioassay method.

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ÖZET
Pamuk yaprakbiti, *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) kimyasal mücadele karşısında hızlı direnç gösteren polifag bir zararlıdır. Direnç yönetiminde direnç düzeylerinin belirlenmesi önemli bir kriterdir. Daha hızlı ve ekonomik bir biyoasay metodunun geliştirilmesi amacıyla, farklı afid populasyonları üzerinde Acetamiprid, Dimethoate, Imidacloprid, L-cyalothrin insektisit etklinlikleri incelenmiştir. Yaprak daldırma metoduyla insektisitler 72 saat, 120 dk süre ve farklı doz uygulamasıyla biyoasaylar yapılarak direnç düzeyleri karşılaştırılmıştır. Her iki metottada 1-10 kat arasında değişen direnç oranları gözlenmiştir. 120 dk'lık hızlı uygulama Acetamiprid, Dimethoate, Imidacloprid, L-cyalothrin insektisitlerine karşı direnç tespitinde hız, uygulama süresi ve metodun ekonomik olması açısından daha avantajlı olup direnç tespitinde ve biyoasay metotlarının geliştirilmesinde katkı sağlayacaktır.

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INTRODUCTION
Cotton aphid *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) is one of the main pests that may cause serious economic losses. It is a polyphagous species with a large host range and is the vector of many viruses (Roistacher et al., 1984). Intensive use of chemicals against this pest causes insecticide ineffectiveness and that results in resistance. It has been reported that it developed multiple resistances against more than 40 active ingredients (Amad et al., 2003; Sparks et al., 2015; Ulusoy et al., 2018). Aphids are capable of rapid reproduction and have a phenomenon called parthenogenetic telescopic generation in which embryos contain embryos (Moran, 1992). This represents millions of clones of aphids reproducing from one aphid in one season (Dixon, 1989; Kersting et al., 1999). Telescopic generations have faster growth rates per unit of time compared to mites (Leather & Dixon, 1984; Dixon, 1989). This effect undoubtedly has an important effect on the development of resistance. The reason is that fast-

growing generations are exposed to more insecticides and develop resistance more quickly (Roush & McKenzie, 1987). Also, these genes do not decrease due to the absence of crossing-over between generations with parthenogenetic reproduction (Wool & Hales, 1997). Considering the biology of this organism, over-spraying and resistance development emerge as an inevitable situation. Resistance levels are a key factor in chemical control and are important not only in terms of the use of prevalence and intensity of insecticide but also in terms of the use of effective control methods. Bioassay methods for this purpose reveal probit curves (log-concentration probit lines) by examining the dose and mortality rates between standard and resistant populations and provide a basis for comparison. Various methods and reports have been studied for insecticide resistance and toxicity tests since the 1970s. In these studies, aphids form exposure to insecticides by direct insecticide contact, by dipping or spraying the leaves of the host plant with different doses of pesticides. Afterward, the dose of mortality rate is determined at different time intervals such as 1, 2, 24, 48 or 72 hours, and the resistance levels are determined as a result of comparison with the reference population (Anonymus, 1979; Hama, 1987; SAS, 1988; Suzuki et al., 1993; McKenzie et al. 1994; IRAC, 2015). Different findings on-time reliability and efficiency were obtained in different bioassay methods tested on *A. gossypii* (Gerami & Heidari, 2013). Due to the easy application of *A. gossypii* bioassays, the method of leaving residue on the leaf as in the IRAC, 2015 is widely preferred. Aphids are small, soft-bodied individuals, and easy damaged during operations such as dipping in pesticides and overflowing. In this method, which is carried out in the form of leaf immersion in some insecticide groups, *A. gossypii* individuals are transported on the leaf discs that have been left with pesticide residue, and the dose-death ratio is determined after 72 hours. It has been reported that bioassay findings may vary depending on nutrition, by taking into account the nutrition, by taking into account the nutritional and starvation condition of the organism in determining the mortality rates after 72 hours (Gerami & Heidari 2013). In addition, revealing the bioassay results of the leaf immersion method after a long period such as 72 hours is a weak point for fast results. The difficulty and reliability of the method is a matter of debate when long periods, feeding of the organism, stabilization of laboratory conditions, economic cost of air conditioning and infrastructure conditions are taken into account. In practice, it has been reported that as the dose increases, the mortality rate and the duration of death are shortened (El Kady et al., 2007; Flores et al., 2007). In this study, by increasing the application dose and shortening the time, the possibility and reliability of performing the current method in a shorter time were tested. For this purpose, Acetamiprid and Imadacloprid from the neonicotinoid group, Dimethoate from the organophosphates group and L-cyalothrin from the pyrethroids group, which are more preferred in the chemical control of *Aphis gossypii*, were used. In determining the level of resistance to these insecticides, the change in the resistance status was revealed by applying normal and high doses at different time intervals with the leaf dipping method. Thus, the result in a shorter time up to 3 hours and the change in resistance levels of the commonly preferred valid method, which results in 72 hours, were examined.

MATERIAL METHOD

Insects; *Aphis gossypii* individuals were randomly collected in three different cotton fields from Adana, Turkey in 2018. The population of the pest was cultured in the climate room at 22± 1 °C, % 60 RH, 12:12 LD photoperiod on cotton plants in Adana Biological Control Research Institute. Wingless adult aphid individuals were used in all studies. *Aphis gossypii* individuals were identified and classified with morphological methods by Dr. Işıl Özdemir at the Directorate Of Plant Protection Central Research Institute, Ankara, Turkey.

The determination of lethal concentrations of Insecticides

The commercial formulations of Acetamiprid, Dimethoate, Imidacloprid, and L-cyalothrin were used during bioassay experiments. IRAC No. 019 (IRAC, 2015) and modifications of this method have been used for this study. Doses, which killed the %95 of the population in 72 hours, were preferred in the first method. In addition, higher doses, which killed %95 of the population in 120 minutes (2 hours), were chosen for the second method. Six different insecticide doses, control were used with 3 replications in this study. The distilled water was used for control. The preparations of insecticide doses were prepared with the mixture of %0.1 tritonX. Petri dishes (30 mm diameter) were used and fresh cotton leaves were cut and placed into Petri dishes. Cotton leaves were dipped in insecticide solution for 15 seconds and dried in a fume hood. According to the first method, leaves were dried for 15-20 minutes in fume hood. According to the second method, leaves were dried for 30-40 minutes except L-Cyalothrin. Due to dense formulations of L-Cyalothrin, leaves dried about 60-70 minutes. Cotton leaves with insecticide were placed into Petri dishes with %1.5 agar. Each Petri dish included 20 wingless aphid individuals. Counting was done 72 hours later for the first method, and 2 hours later for the second method differently. The lowest LC_{50} levels of populations were determined as susceptible for the determination of the resistance rate between populations.

The determination of lethal time levels

According to the results of two bioassay methods of LC_{50} values, a higher one-dose application was done to all populations and LT_{50} levels were detected. 40 ppm (0.04 mg/L) and 350 ppm (0.35 mg/L) for Acetamiprid, 12 ppm (0.012 mg/L) and 120000 ppm (120 mg/L) for Dimethoate, 40 ppm (0.04 mg/L) and 40000 ppm (40 mg/L) for Imidacloprid, 100 ppm (0.1 mg/L) and 40000 ppm (40 mg/L) for L-cyalothrin were applied during normal and higher dose experiments. The counting was done 0., 9., 12., 32., 46., 56. and 72. hours for the first method and other counting were done at 30., 60., 75., 90. and 120. minutes for the second method.

Statistical analysis

LC_{50} and LT_{50} Dose, and Time-response regressions were computed using Polo-Plus computer program (LeOra Software, Berkeley, CA, USA). To estimate the LC_{50} (lethal dose to kill 50% of the test population), resistance factors were calculated by dividing the LC_{50} of the field-collected population by the LC_{50} of the susceptible population.

RESULT and DISCUSSION

Bioassay LC_{50} analysis results obtained by leaf dipping method are given in Table 1. In the bioassay tests applied against four insecticides, the lowest LC_{50} values were observed in the 3rd population. In the determination of the resistance rates, the 3rd population was taken as a reference as a susceptible population. RR50 values were found by proportioning the LC_{50} values of the resistant population to susceptible population in both methods where the count was made after 72 hours and 2 hours (120 minutes) (Table 1). Although this rate varies according to the insecticide type, it was found as 1.15 times in the lowest imidacloprid and 2.82 times in the highest acetamiprid in the 1st population. In method II the IRAC method was tested by modifying, it was observed that the most sensitive population was again the 3rd population in parallel with the first method. Since high doses were used in the method II, LC_{50} levels were observed at a very high rate. When the resistance ratios were examined, the population no 2 had the lowest resistance rate against dimethoate at 1.11 times, and the population of the no 1 had the highest resistance rate at 2.61 times. Although the RR_{50} ratios vary in both methods, it was seen that the data were close to each other. With a single dose application, probit curves were drawn and LT_{50} levels were calculated by counting at different times in both methods, (table 2). In the first method where lower doses were applied, the fastest death occurred in population 3 with 11.3 hours. It has been found that L-cyalothrin had the slowest mortality rate in population 2 with 37.2 hours. In the method II, the lowest LT_{50} rate was observed in acetamiprid with 32.3 min, and the highest LT_{50} level was observed in imidacloprid with 66.7 min., the variation of mortality rates of populations with different insecticides at constant dose versus time was plotted in the method I and method II (figure 1). Bioassays of different groups of insecticides were tested on *A. gossypii* populations by leaf dipping method at different doses and time intervals. As a result of the comparison of the resistance ratios of the two methods, the resistance ratio changed between 1 to 2 fold. LC_{50} and RR_{50} results were revealed (table 1). It was observed that the resistance rate of acetamiprid decreased from 2.82 to 1.85 for the 1st population between the two methods while the dimethoate increased from 1.83 to 2.61 and there was a proportional difference of approximately 1.5 times. For other insecticides, it was found that these ratios vary between 0.7 and 1.3. When the relationship between resistance rate and resistance indicator was examined in different studies, it was seen that they had close approaches. Leong et al., 2020, reported that a resistance range of 1-10 times would not be considered resistant in the management against woodworms and insecticides. It also reported that a range of 1-10 times would not be considered resistant, similar to the resistance scale made in mites (Kim et al., 2004; Martínez et al., 2021). It has been revealed that the management of citrus fruits and mealybugs will not be resisted at a range of 1-10 times (Venkatesan et al., 2016). In resistance studies, the LC_{50} value of the susceptible population is an important reference in demonstrating the necessity of resistance management. In this study, the changing levels of the resistance rates obtained in the 72-hour and 120-minute leaf dipping bioassay methods were revealed. Considering the previous references, it has been found that there was a resistance ratio between 1 and 10 times in common in both methods. When the sensitive population was taken as a reference, it was understood that there was no effective resistance to the insecticides used among these populations. Although the sensitivity of the sensitive population shows the possibility of revealing a different situation related to the level of resistance, it has been concluded that there will be no change in the resistance rates by applying the two methods to all populations at the same time, that is, the results are homogeneous. When LT_{50} ratios were examined, it was found that the same insecticides had a faster lethal effect depending on time at higher doses (table 2, figure 1). different insecticides have different LT_{50} durations and considering the mechanism of action and physiological effects of insecticides on insects in addition to higher doses will provide a more accurate conclusion. In addition, it was observed that the LT_{50} levels of the population no 3, which were thought to be susceptible, were higher in 72-hour applications of acetamiprid and dimethoate compared to the population no 1 and 2, which were thought to be resistant. In the emergence of this

situation, it can be concluded that population has a heterogeneous population structure arising from individuals reproducing clonally and having identical or very similar genetic structures, it has been reported in different sources that physiological changes in metabolic and enzyme levels may cause this heterogeneity in death time (Field et al., 1999; Ranson & Hemingway, 2005; Li et al., 2007; Bass & Field, 2011). When comparing the toxic effects of insecticides in terms of duration, more accurate results can be obtained by comparing the mortality time per unit active substance over homogeneous populations. Gerami & Heidari, (2013), showed that mortality changed, in terms of reliability in determining LC_{50} rates, depending on the prolongation of time and fasting status in bioassay trials.

Table 1. LC_{50}, LC_{90} values rates of Acetamiprid, Dimethoate, Imidacloprid, L-cyalothrin of Aphis gossypii
Çizelge 1. *Aphis gossypii populasyonu Acetamiprid, Dimethoate, Imidacloprid, L-cyalothrin LC_{50}, LC_{90} değerleri*

Insecticide	n	P	LC_{50}(ppm) (CI)	LC_{90}(ppm) (CI)	Slope (± SE)	X^2	RR_{50}
72 h exposure							
	380	No:1	20.39 (11.98-31.98)	117.78 (62.72- 560.70)	1.68±0.40	1.68	***2.82***
Acetamiprid	368	No:2	9.54 (4.00-16.50)	83.48 (38.92-723.6)	1.36±0.37	1.73	***1.32***
	370	No:3	7.22 (2.56-12.47)	62.64 (30.40-502.20)	1.36±0.38	0.18	-
	360	No:1	5.68 (2.15-9.44)	26.42 (14.72-142.69)	1.92±0.35	5.05	***1.83***
Dimethoate	370	No:2	5.55 (2.29-8.32)	25.38 (17.11-59.05)	1.94±0.48	2.99	***1.79***
	374	No:3	3.10 (0.44-5.45)	14.10 (9.22-31.83)	1.95±0.60	1.81	-
	368	No:1	13.18 (5.22-23.68)	164.73 (66.44-350.12)	1.16±0.33	1.56	***1.15***
Imidacloprid	380	No:2	19.39 (8.71-39.51)	272.68 (92.76-648.23)	1.11±0.34	1.16	***1.69***
	390	No:3	11.43 (3.12-32.59)	155.32 (167.82-62905)	0.73±0.20	0.28	-
	360	No:1	32.33 (3.88-63.38)	763.20 (282- 1200.4)	0.93±0.30	0.53	***1.25***
L-Cyalothrin	380	No:2	40.14 (8.99-65.9)	212.65 (133.3-786.3)	1.77±0.53	1.18	***1.55***
	370	No:3	25.77 (5.11-47.1)	317.03 (161.2-522.9)	1.17±0.33	2.71	-
120 min exposure							
	380	No:1	289.84 (154.26-420.20)	1372.51 (939.22- 2652.61)	1.89±0.37	0.75	***1.85***
Acetamiprid	368	No:2	215.60 (86.96-339.05)	1164.34 (780.13-2347.71)	1.75±0.38	1.34	***1.37***
	370	No:3	156.61 (39.47-280.68)	1090.09 (696.29-2354.08)	1.52±0.36	1.19	-
	360	No:1	84471.89 (64542.32-124817.1)	309201.30 (178919.9-816627.8)	2.274±0.61	0.14	***2.61***
Dimethoate	370	No:2	35935.75 (10873.14-51712.16)	184359.71 (112170.89-340322.5)	1.80±0.59	0.05	***1.11***
	374	No:3	32360.61 (13008.25-45100.4)	121720.29 (85683.50-333523.65)	2.22±0.64	0.64	-
	368	No:1	17183.59 (8650.4-38509.1)	88085.63 (39066-162879.1)	1.80±0.31	6.66	***1.07***
Imidacloprid	380	No:2	21643.53 (11315.4-53295.8)	90214.33 (41238.6-178060.1)	2.06±0.33	7.82	***1.35***
	390	No:3	15948.54 (8718.8-63839.6)	86569 (33746.9-1052308)	1.82±0.36	3.73	-
	360	No:1	15570.85 (10431.24-32278.9)	136893.5 (51350.5-268833.7)	1.35±0.42	0.49	***1.36***
L-Cyalothrin	380	No:2	16336.61 (11386.7-31338.5)	117399.36 (48999.4-242368.8)	1.49±0.43	0.61	***1.42***
	370	No:3	11447.45 (7344.3-17860.4)	86170.64 (39051.9-105588.9)	1.46±0.42	0.04	-

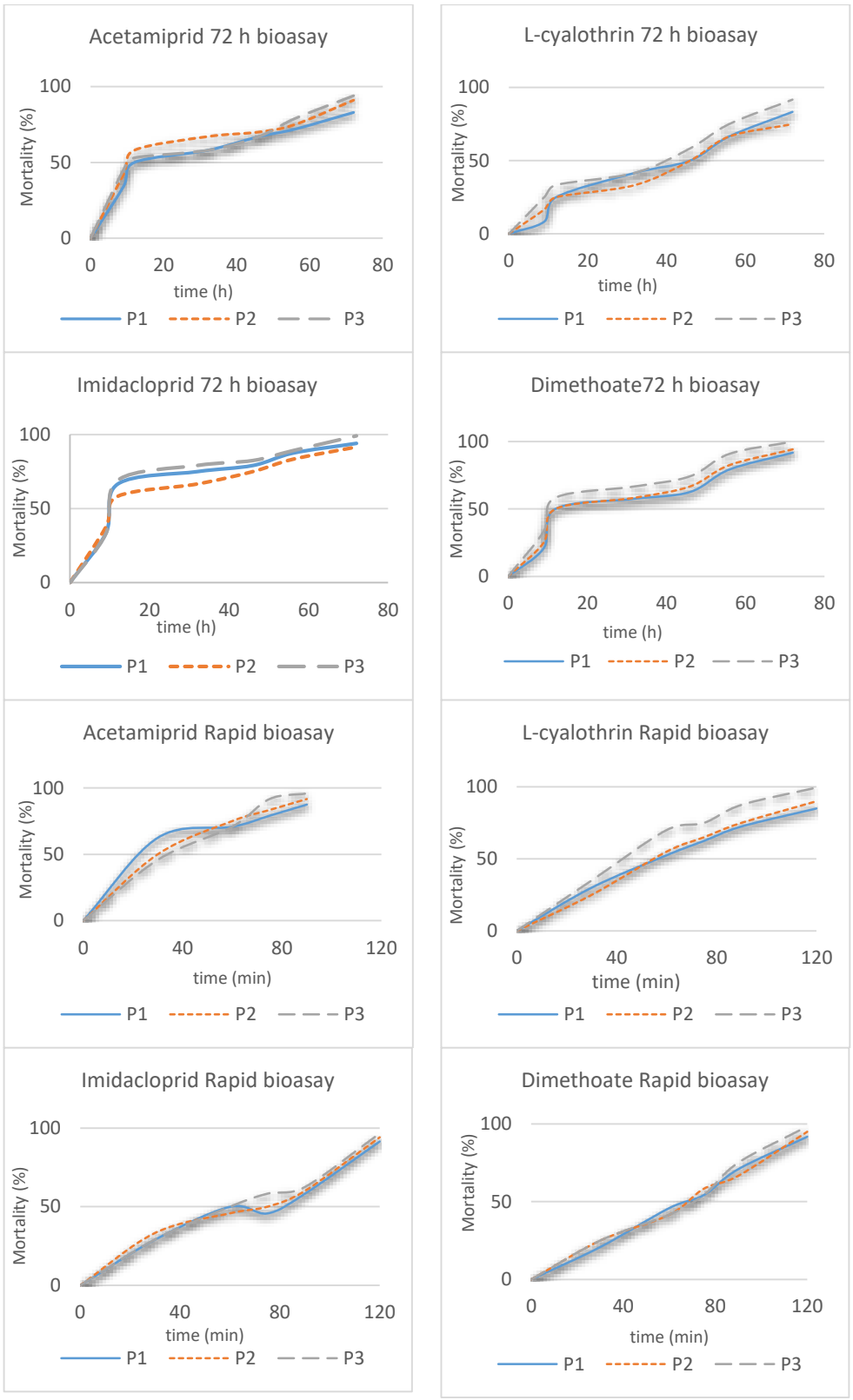

Figure1. Different A. gossypii populations mortality-time slope at different insecticide doses
Şekil 1. A. gossypii populasyonlarının farklı insektisit dozlarında ölüm-zaman değişimi

Table 2. LT_{50} values of Acetamiprid, Dimethoate, Imidacloprid, L-cyalothrin of Aphis gossypii
Çizelge 2. Aphis gossypii populasyonu Acetamiprid, Dimethoate, Imidacloprid, L-cyalothrin LT_{50} değerleri

Insecticide	P	LT_{50}(hour) (CI)	Slope (± SE)	X^2	LT_{50}(minute) (CI)	Slope (± SE)	X^2
		72 hour			120 minute		
Acetamiprid	No:1	16.59 (4.85-27.4)	1.41±0.46	1.73	32.36 (11.24-44.97)	3.10±0.99	0.88
	No:2	14.51 (3.62-24.10)	1.42±0.37	2.76	37.76 (19.77-49.46)	3.51±0.99	1.25
	No:3	17.09 (9.09-24.82)	2.00±0.50	3.40	35.33 (19.57-45.40)	4.22±1.23	1.22
Dimethoate	No:1	16.59 (4.85-27.40)	1.41±0.35	1.73	56.73 (35.57-74.71)	2.93±0.90	1.14
	No:2	19.79 (10.76-29.10)	1.90±0.49	3.10	59.08 (38.86-77.77)	2.99±0.91	1.60
	No:3	18.02 (8.29-27.51)	1.71±0.48	2.28	55.51 (36.83-71.21)	3.24±0.92	1.34
Imidacloprid	No:1	11.39 (4.47-17.38)	1.99±0.53	2.80	63.32 (41.62-87.03)	2.77±0.89	2.49
	No:2	13.33 (3.67-21.74)	1.53±0.48	1.35	66.72 (48.94-87.10)	3.34±0.96	1.82
	No:3	11.06 (3.12-17.85)	1.72±0.50	1.77	52.52 (25.09-72.26)	2.47±0.87	1.61
L-Cyalothrin	No:1	33.18 (21.20-54.37)	1.80±0.49	1.53	50.39 (32.60-63.86)	2.55±0.67	0.18
	No:2	37.27 (23.48-69.43)	1.65±0.48	1.44	52.57 (38.23-64.34)	1.49±0.43	0.61
	No:3	25.24 (14.53-39.10)	1.74±0.48	3.08	40.73 (27.03-50.68)	3.26±0.73	0.66

CONCLUSION and RECOMMENDATIONS

Obtaining faster and more reliable results is an important factor in studies on resistance. The need for air conditioning for 72 hours compared to 120 minutes is another disadvantage of the method in terms of economy. Consequently, This study demonstrated the possibility of revealing resistance rates of the *A. gossypii* against different insecticides in as little as 120 minutes and will help to contribute to the development of faster and more economical bioassay methods.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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