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RESEARCH PAPER

HORTICULTURAL STUDIES

Determination of Insecticide Resistance in Western Flower Thrips [*Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae)] Causing Problems in Carnation Cultivation

Musa KIRIŞIK[1] **Tuba KAHRAMAN[1]**

[1]Batı Akdeniz Agricultural Research Instiute, 07100, Antalya, Türkiye

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Corresponding Author
E-mail:musa.kirisik@tarimorman.gov.tr

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Abstract

The fact that *Frankliniella occidentalis* (Thysanoptera: Thripidae) completes its life cycle in a short time reveals the need for continuous pest control. Therefore, pest resistance may occur with the intensive use of chemicals by the growers. In this context, the current sensitivity of *F. occidentalis* to registered insecticides for carnation (methiocarb, formetanate hydrochloride), registered insecticides for vegetables (azadirachtin, malathion, and spinosad), and unapproved insecticides used extensively by growers needs to be determined. Moreover, it is important to test the chemicals (for example, pyridalyl) used against other pests whose spray period is the same as *F. occidentalis*. In this study, it was aimed to determine the sensitivity levels of *F. occidentalis* populations taken from the sites of intensive carnation production in Antalya province in 2018-2020 to these chemicals by the leaf dipping method. As a result of the study, resistance against spinosad (11.00-28.60 times), methiocarb (2.10-2.70 times), malathion (2.05-4.21 times), azadirachtin (3.00-7.00 times), formetanate hydrochloride (1.50-2.00 times) and pyridalyl (2.75-3.89 times) were determined. Given the high resistance to spinosad observed in the study, trials involving the combination of spinosad and formetanate hydrochloride were initiated as a strategy for managing resistance. The resistance against spinosad + formetanate hydrochloride was determined between 4.35 and 9.09 times. Our results suggest that resistance level can be reduced by using resistance management methods such as the use of mixed chemicals, although resistance was detected in all five locations against all active substances.

1. Introduction

Türkiye has a significant advantage in the cultivation of ornamental plants due to its convenient location in terms of both climatic and geographical conditions. Carnation, rose, and gerbera are the leading species in cut flower production. Their share in the total cut flower production areas is 63%, 15%, and 10%, respectively. The greenhouse area constitutes two-thirds of the total production area. Furthermore, most of the production in greenhouses is for export (Anonymous, 2016).

Spider mite, thrips, and cotton bollworm are among the main pests in carnation, however, the most important among them in Antalya province is the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera, Thripidae) (Keçecioğlu and Madanlar, 2002). This pest, whose origin is North America, was first detected in Türkiye in 1993 in carnation greenhouses in Antalya and its host range is very wide (Tunç and Göçmen, 1995). The western flower thrips, and the onion thrips *Thrips tabaci* (Lindeman) (Thysanoptera, Thripidae) are economically important pests in many cultivated plants, including ornamental plants. These two thrips species are vectors of Tomato spotted wilt virus (TSWV) and Impatiens Necrotic Spot Virus (INSV) virus, which are also a problem in ornamental plants and cause significant problems in production areas (Daughtrey et al., 1997; Ulmann et al., 1997). Dağlı and Tunç (2006) stated in their study that pesticides are widely used against *F. occidentalis* in Antalya province, that growers do not consider chemical control to be sufficient, and that this may be due to resistance developed by *F. occidentalis*. The pest is protected from insecticide applications because it lays its eggs into plant tissue, the adults and larvae are in the inner parts of the flowers, and the pupa is in the parts that are protected from pesticides on the soil or plant (Robb and Parella, 1995). In addition to these biological properties of *F. occidentalis*, it has been demonstrated in many studies that resistance to most of the different insecticide groups makes chemical control increasingly difficult (Immaraju et al. 1992; Brodsgaard, 1994; Zhao et al. 1995; Karadjova, 1998; Kontsedalov et al. 1998; Jensen, 2000; Espinosa et al., 2002; Herron and James, 2005; Bielza et al., 2007).

In this study, the current resistance status of methiocarb and formetanate hydrochloride licensed to *F. occidentalis* in carnation against populations obtained from Antalya carnation areas was determined. Moreover, as it is known in Antalya, greenhouse vegetable production is carried out intensively. Since vegetable production areas and ornamental plant production areas are very close to each other, pests can easily be transmitted from one production area to another. Furthermore, *F. occidentalis* is a pest that can be found in vegetable and ornamental plant production areas and can easily pass from one greenhouse to another. Although malathion, azadirachtin, and spinosad used in this experiment are not licensed for *F. occidentalis* in carnation, they were selected because they are licensed for thrips species in vegetables. The other selected active ingredient, pyridalyl, is licensed against *Helicoverpa armigera* and *Tuta absoluta* in vegetable greenhouses, and the application time coincides with the thrips control time. The determination of the resistance status of *F. occidentalis* against pyridalyl was also included in the experiment.

The aim of the study was to determine the current resistance status of *F. occidentalis* against malathion, methiocarb, azadirachtin, formetanate hydrochloride, pyridalyl, and spinosad and to conduct studies on resistance management if resistance is detected.

2. Materials and Methods

2.1. Materials

In the study, malathion, methiocarb, formetanate hydrochloride, and spinosad, which are commonly used in the control of *F. occidentalis*, as well as pyridalyl and azadirachtin that are licensed for carnation in other countries were discussed. During the bioassay (chemical tests) studies, laboratory materials such as micropipette, disposable plastic Petri dishes, disposable plastic cups, cotton, thin and soft-tipped brush, soft-tipped forceps, 100 ml measuring cylinder, 10 ml pipette, and gloves were used.

2.2. Collection of *Frankliniella occidentalis* populations

Samples of *F. occidentalis* were taken from Aksu, Kepez (Altınova), and Serik districts, where greenhouse carnation cultivation is most common in Antalya (Table 1). In the collection of samples, attention was paid to take samples from the production areas of private sector companies that produce carnations for export. The sensitive population was obtained from Assoc. Prof. Fatih Dağlı (Akdeniz University, Faculty of Agriculture, Department of Plant Protection, Antalya, Türkiye). The samples taken were produced on green bean pods at the climate room of Plant Protection Department of BATEM (Batı Akdeniz Agricultural Research Institute, Antalya, Türkiye) with a temperature of 24±1ºC and a day length of 16:8 h (light: dark).

2.3. Insecticides used in the study

The active ingredients, trade names, formulations and IRAC mode of action classification of the insecticides used in the study are given in Table 2.

2.4. Thrips diagnosis and production

Species identification of populations was made by PhD. Emine Topuz using morphological diagnostic criteria. Green bean pods were used to provide nutrition and egg laying environment for thrips. Plastic containers with lids covered with filter paper were used as production cages and 2-3 layers of paper towels were laid on the bottom of the containers for thrips to pass the pupal stage. Fresh bean pods, which were disinfected with bleach,

Table 1. Places and times of collection of samples.

Cooperated carnation exporter companies	Sample collection date
Antalya Tarım Company Limited (Aksu)	September 19, 2017
Flash Tarım Company Limited (Kepez)	September 19, 2017
Tempo Tarım Company Limited (Kepez)	October 24, 2017
Erkut Tarım Company Limited (Kepez)	October 24, 2017
Ada Tarım Company Limited (Serik)	October 24, 2017

Table 2. Insecticides used in the study, their trade names and mode of action.

Active ingredient	Trade name, formulation, *recommended dose	**IRAC mode of action classification
Methiocarb	Mesurol WP 50% Bayer 1000 ml ha^{-1} (100 L water)	Carbamate (1A), acetylcholinesterase inhibitor in the nervous system
Formetanate hydrochloride	Dicarzol 50 SP 500 g kg^{-1} AMC –TR 100 g 100 L^{-1} water	Carbamate (1A), acetylcholinesterase inhibitor in the nervous system
Spinosad	Laser SC 480 g L^{-1} Dow Agro Sciences, 200 ml ha^{-1} (100 L water)	Spinosyn (5), activating effect on nicotinic acetylcholine receptors in the nervous system
Malathion	Malathion EC 650 g L^{-1} Sefa, 100 ml 100 L^{-1} water	Organophosphate (1B), acetylcholinesterase inhibitor in the nervous system
Pyridalyl	Sumipleo 50 EC 500 g L^{-1} Sumiagro 200 ml ha^{-1}	Unknown
Azadirachtin	Nimbecidine EC 0.3 g L^{-1} Agrobest 500 ml 100 L^{-1} water	Unknown

* Recommended dosage according to label information, **IRAC: Insecticide Resistance Action Committee.

dipped in sugar and amino acid solutions and dried, were left in the production cages together with the thrips desired to be produced, and they were replaced with new ones at 3-4 day intervals.

2.5. Insecticide tests

For insecticide tests, the method of Contreas et al. (2008) was adapted and the leaf dipping method was used (IRAC Test Method: 1068). To determine the lethal concentration (LC) values for *F. occidentalis* population, at least six different dose series were prepared to create a death distribution between 0% and 100%. In addition, only water (+Tx-100) was used in the controls. Cowpea leaf discs with a diameter of 3 cm were immersed in the prepared insecticide concentrations for 5 seconds and dried, and then placed in Petri dishes on which agar was poured. Adult female thrips collected from production containers with a mouth aspirator were transferred to the Petri dishes by temporarily stunned with carbon dioxide, and finally, the top of the stretch film was punctured with an insect needle to allow air to breathe. After the treated thrips were exposed to insecticide residues for two days in the Petri dishes, mortality counts were made.

Obtained mortality rates were subjected to probit analysis and LC_{50}, LC_{90}, and LC_{99} values were determined. In this test, at least four replications for each different concentration and at least 20-25 female thrips adults were used for each replication. Resistivity multiples were determined by comparing sensitive population LC values with all populations.

2.6. Location of the project

The fieldwork in the experiment was carried out in the regions where carnation is produced intensively in Antalya, which is indicated in Table 1. Laboratory studies were carried out in the entomology laboratories of the Batı Akdeniz Agricultural Research Institute (BATEM), Antalya, Türkiye.

2.7. Statistical analysis

In all populations, LC_{50}, LC_{90} and LC_{99} values, slopes and 95% confidence intervals of the populations were obtained by using the probit analysis method in the POLO computer package program (LeOraSoftware, 2008) by using the determined mortality rates.

3. Results and Discussion

The LC values of active substances for *F. occidentalis* populations obtained from Antalya Tarım, Tempo Tarım, Flaş Tarım, Erkut Tarım, and Ada Tarım populations as a result of the bioassay studies are given in Table 3.

The highest resistance rates were determined in the Ada Tarım population with 28.6 times to spinosad, 2.7 times to methiocarb, 4.2 times to malathion, 7.0 times to azadirachtin, 2.0 times to formetanate hydrochloride, and 3.89-times to pyridalyl. The dose level that kills 99% of the resistant population (LC_{99}) in laboratory tests was still higher than the practically recommended dose of spinosad and methiocarb. The results show that applications of spinosad and methiocarb against this pest may fail in the locations where these populations are sampled. With additional research, the prevalence of spinosad and methiocarb resistance throughout the country should be revealed, and the effective life of these insecticides should be tried to be extended by allowing them to be applied for limited times in locations without resistance problems.

However, in this study, it was determined that there was no resistance problem in the sample locations by looking at the LC_{99} values of azadirachtin and formetanate hydrochloride (except Ada Tarım). The reason for identifying pyridalyl-resistant populations is thought to be that it is used to control cotton bollworm in carnations, thus indirectly affecting the thrips populations. The

Table 3. The resistance level of *Frankliniella occidentalis* populations to active substances.

Population	Active ingredients	n	slope±se	LC_{50} mg(a.i.)/l (95% confidence limit)	* Sensitivity differences according to LC_{50} values (=resistance multiples)	LC_{90} mg(a.i.)/l (95% confidence limit)	LC_{99} mg(a.i.)/l (95% confidence limit)	Field recommended dose mg (a.i)/l
Sensitive	Spinosad	401	1.5±0.3	1.4 (0.6-3.1)	-	2.6 (1.4-4.3)	3.1 (1.8-6.8)	96
	Methiocarb	401	1.4 ±0.3	32.8 (19.9-41.1)	-	81.6 (62.4-95.4)	115.1 (96.5-136.5)	500
	Malathion	406	2.0±0.3	99.8 (81.4-115.3)	-	397 (366.4-465.3)	615 (591.4-665.3)	650
	Azadirachtin	401	1.6±0.2	0.1 (0.04-0.3)	-	0.9 (0.4-1.4)	1.2 (0.04-0.3)	1.5
	Formetanate hydrochloride	401	1.4±0.1	40.3 (29.6-59.8)	-	120.5 (102.9-144.3)	180.6 (162.3-204.7)	500
	Pyridalyl	401	1.5±0.2	15.4 (8.5-24.0)	-	32.1 (11.5-53.5)	41.8 (22.6-69.9)	100
	Spinosad+Formetanate hydrochloride	399	1.7±0.3	1.2 (0.5-2.4)	-	2.4 (1.4-3.5)	2.8 (1.3-4.4)	96+500
Antalya Tarım	Spinosad	413	1.4±0.2	32.4 (21.4-65.3)	23.10	125.6 (110.4-295.7)	175.2 (152.2-301.6)	96
	Methiocarb	409	1.5±0.2	70.5 (49.3-89.8)	2.10	513.9 (425.5-998.2)	632.4 (532.4-1024.8)	500
	Malathion	402	1.8±0.2	205.4 (181.4-225.3)	2.05	525.6 (500.4-695.7)	645.2 (632.2-701.6)	650
	Azadirachtin	399	1.5±0.2	0.6 (0.2-1.0)	6.00	1.0 (0.8-1.3)	1.1 (0.9-1.4)	1.5
	Formetanate hydrochloride	408	1.3±0.2	60.4 (39.3-89.8)	1.50	323.9 (305.6-348.2)	436.6 (405.4-464.8)	500
	Pyridalyl	398	1.6±0.3	42.4 (31.4-63.3)	2.75	75.6 (60.4-95.3)	95.6 (90.4-112.7)	100
	Spinosad+Formetanate hydrochloride	403	1.8±0.3	12.4 (8.6-17.2)	10.33	30.4 (22.3-38.4)	41.8 (28.3-66.3)	96+500
Flash Tarım	Spinosad	401	1.3±0.1	26.8 (16.5-52.8)	19.10	132.9 (72.4-183.7)	176.1 (132.8-298.5)	96
	Methiocarb	401	1.4±0.2	86.3 (53.2-96.5)	2.60	550.7 (433.2-957.6)	701.6 (648.6-1357.3)	500
	Malathion	400	1.5±0.1	260.8 (235.5-292.8)	2.61	632.9 (572.4-683.7)	698.1 (638.8-728.5)	650
	Azadirachtin	412	1.3±0.1	0.5 (0.2-0.9)	5.00	0.9 (0.7-1.4)	1.1 (0.8-1.4)	1.5
	Formetanate hydrochloride	399	1.4±0.3	76.3 (55.6-96.9)	1.90	350.7 (333.2-397.3)	455.6 (406.3-487.2)	500
	Pyridalyl	411	1.9±0.4	46.8 (36.5-72.8)	3.03	82.9 (67.2-93.4)	92.9 (72.5-123.7)	100
	Spinosad+Formetanate hydrochloride	415	1.9±0.3	16.7 (12.6-26.8)	13.91	56.5 (48.3-71.4)	72.5 (50.6-95.4)	96+500
Tempo Tarım	Spinosad	395	1.4±0.1	15.4 (8.5-24.0)	11.00	112.4 (86.8-221.3)	148.5 (113.7-242.6)	96
	Methiocarb	395	1.3±0.1	85.6 (52.3-100.2)	2.60	541.3 (312.5-967.6)	687.1 (624.7-1101.6)	500
	Malathion	398	1.3±0.1	346.8 (308.5-424.0)	3.47	612.4 (586.8-641.3)	648.5 (599.7-675.6)	650
	Azadirachtin	395	1.4±0.2	0.5 (0.1-0.9)	5.00	1.0 (0.6-1.3)	1.2 (0.5-1.5)	1.5
	Formetanate hydrochloride	396	1.8±0.4	65.6 (52.3-79.2)	1.60	381.3 (354.3-412.1)	486.1 (422.7-500.6)	500
	Pyridalyl	405	1.8±0.4	49.3 (42.0-75.5)	3.20	105.4 (76.2-121.5)	112.4 (96.8-131.3)	100
	Spinosad+Formetanate hydrochloride	404	2.0±0.5	12.3 (8.1-19.2)	10.25	34.3 (25.2-51.6)	48.6 (30.8-72.2)	96+500
Erkut Tarım	Spinosad	408	2.0±0.3	17.3 (12.0-25.5)	12.40	102.3 (76.3-210.8)	135.6 (101.2-220.5)	96
	Methiocarb	411	1.4±0.1	78.7 (51.0-93.7)	2.40	516.8 (432.6-1012.3)	650.8 (554.2-1142.7)	500
	Malathion	405	1.7±0.2	273.3 (212.0-298.5)	2.73	502.3 (476.3-530.8)	635.6 (601.2-670.5)	650

*: LC_{50} value of populations / LC_{50} value of the most susceptible (lowest LC_{50}) population.
a.i.: active ingredient, n: number of pests used in the test.

Table 3. The resistance level of Frankliniella occidentalis populations to active substances (contin.).

Population	Active ingredients	n	slope±se	LC_{50} mg(a.i.)/l (95% confidence limit)	* Sensitivity differences according to LC_{50} values (=resistance multiples)	LC_{90} mg(a.i.)/l (95% confidence limit)	LC_{99} mg(a.i.)/l (95% confidence limit)	Field recommended dose mg (a.i)/l
Erkut Tarım	Azadirachtin	402	2.2±0.3	0.3 (0.2-0.6)	3.00	0.8 (0.4-1.1)	1.1 (0.8-1.5)	1.5
	Formetanate hydrochloride	400	1.5±0.3	78.7 (62.1-88.6)	1.90	366.8 (332.3-402.1)	474.8 (439.1-498.7)	500
	Pyridalyl	406	2.1±0.3	57.3 (42.0-75.5)	3.72	112.3 (86.4-132.5)	132.3 (96.3-150.8)	100
	Spinosad+Formetanate hydrochloride	398	1.6±0.2	14.2 (10.3-21.2)	11.83	55.1 (46.4-68.4)	72.3 (57.3-96.2)	96+500
Ada Tarım	Spinosad	411	2.1±0.1	40.0 (25.4-72.8)	28.60	140.8 (88.5-261.7)	195.9 (147.6-320.2)	96
	Methiocarb	406	2.0±0.3	90.5 (59.3-139.8)	2.70	620.6 (389.5-1212.3)	745.6 (701.5-1443.2)	500
	Malathion	403	2.1±0.1	420.2 (375.4-472.8)	4.21	640.8 (588.5-661.7)	715.9 (647.6-745.2)	650
	Azadirachtin	400	2.3±0.4	0.7 (0.3-0.9)	7.00	1.3 (0.9-1.4)	1.5 (1.0-1.8.)	1.5
	Formetanate hydrochloride	403	2.1±0.4	80.5 (62.9-96.3)	2.00	389.6 (338.5-415.3)	506.9 (471.5-543.6)	500
	Pyridalyl	402	2.3±0.2	60.0 (45.4-82.8)	3.89	120.8 (98.6-144.3)	140.8 (121.5-161.7)	100
	Spinosad+Formetanate hydrochloride	403	1.9±0.3	31.1 (20.2-40.6)	25.91	80.7 (71.3-98.6)	102.4 (86.5-132.5)	96+500

*: LC50 value of populations / LC50 value of the most susceptible (lowest LC50) population.
a.i.: active ingredient, n: number of pests used in the test.

reason for malathion resistance can be interpreted as the fact that the active ingredients has been used in the control of many pests, including thrips, for many years.

As a result of the study, the highest resistance ratios were observed in spinosad active ingredient. In spinosad's screening for resistance in populations, the lowest resistance rate was determined to be 11.0 times and the highest was 28.6 times. Furthermore, the lowest resistance ratio was observed in formetanate hydrochloride active ingredient with 1.5 times and the highest with 2 times. In the last year of the study, a lower resistance rate was obtained in the spinosad+formetanate hydrochloride mixture test compared to the spinosad application alone test. As a result of this experiment, it was determined that two insecticide mixtures with different modes of action caused a decrease in the resistance of spinosad-resistant populations. It is predicted that this application can be recommended for resistance management in locations with spinosad resistance.

The results obtained from the resistance studies should also give an idea about the level of negative impact on the success of pesticide applications in the field. Considering this situation, the resistance of the populations collected from the field was compared to the susceptible population in our study. Besides, the dose values (LC_{99}) that killed 99% of the field populations were compared with the recommended dose values of the insecticides in question in the field. Although the laboratory results do not reflect the practice exactly, the insecticide test method we used in the study shows a close resemblance to the practice. In our tests, thrips are exposed to the chemical under the most severe conditions. The pests are forced to feed for two days in the chemically treated area on a real leaf disc (without the possibility of escape). Chemicals that are ineffective under these conditions may have great difficulty succeeding under field conditions. From this point of view, if the LC_{99} dose value of an insecticide obtained in laboratory tests against a population is higher than the field-recommended dose of this chemical, it should be considered that the pesticide applications to be made in the field may fail.

Flower thrips have the potential to develop significant resistance to insecticides used for the control of *F. occidentalis*. Studies conducted in America, Australia, European countries, and Israel have reported that the species in question develop varying levels of resistance to the following chemical classes: endosulfan in the cyclodiene group; acephate, chlorpyrifos, malathion, dichlorvos, dimethoate, monocrotophos, and methamidophos in the organophosphorus group; methiocarb, methomyl, carbosulfan, and formetanate in the carbamate group; acrinathrin, bifenthrin, and cypermethrin in the pyrethroid group, abamectin in the macrocyclic lactone group, spinosad in the spinosyn group (Immaraju et al., 1992; Brodsgaard, 1994; Zhao et al., 1995; Karadjova, 1998; Kontsedalov et al., 1998; Jensen, 2000; Espinosa et al., 2002; Herron and James, 2005; Bielza et al., 2007; Dağlı and Tunç, 2007).

Bielza et al. (2007) indicated that the LC_{50} values of *F. occidentalis* samples collected from areas in Murcia where no spinosad application had been made before, were between 0.005-0.077 mg l^{-1} and were sensitive. Moreover, the samples collected from Almeria were resistant to spinosad when compared with the sensitive population of Bielza. In addition, it was determined in the study that spinosad did not have cross-resistance with methiocarb, acrinathrin and formetanate. Besides, Dağlı et al. (2010) determined methiocarb resistance in populations taken from Antalya and its districts between 1.4-15.3 times according to LC_{50} values. It was stated that applications with methiocarb may fail at all points where the populations have been taken. Dağlı et al. (2010) determined resistance to spinosad in populations taken from Antalya and its districts between 1.0-141 times according to LC_{50} values. It was stated that applications with spinosad may fail at all points where the populations have been taken. Furthermore, Dağlı (2018) investigated spinosad resistance in *F. occidentalis* population from Antalya and determined a 235-times loss of sensitivity in the population compared to LC_{50} values. However, in this study, spinosad resistance of populations was determined as 11.0-28.6 times. The reason for the resistance multiples difference between the present study and Dağlı (2018) is thought to be the presence of vegetable fields around the carnation greenhouses and the transition of possible sensitive populations to the carnation greenhouses.

Espinosa et al. (2002) reported 0.5-23.0-times loss of sensitivity to Formetanate and 1.3-22.3 times to methiocarb in *F. occidentalis* populations collected from Spain. However, in this study, a 1.5-2 times loss of sensitivity to Formetanate and a 2.1-2.7 times loss of sensitivity to methiocarb was detected. In another study, Jensen (1998) reported 9.5 times resistance to Methiocarb in the *F. occidentalis* population. Espinosa et al. (2002) determined an approximate 10 times reduction between Formetanate and methiocarb compared to this study. The fact that there are different vegetable areas around and the populations are crossing each other is considered to be the reason for the loss of sensitivity in the populations. Another important result of our study was that applications with spinosad+formetanate hydrochloride against this pest in locations known to have spinosad resistance could reduce the resistance.

4. Conclusion

Economic and environmental losses can be prevented to some extent by avoiding excessive and unnecessary insecticide applications against resistant populations. Studies yet to be conducted should regularly monitor sensitivity to any chemicals that may be used alternatively to control *F. occidentalis* field populations. Moreover, with comprehensive research on populations, cross or multiple resistance spectra for current insecticides, resistance mechanisms and the inheritance pattern of resistance (genetics) should be revealed and basic resistance management programs should be established. Both in this study and other studies on resistance screening, it has been proven that the chemical-based control method becomes ineffective after a while due to resistance. At this point, it is imperative to expand the practical use of other control methods (such as cultural, biological, and biotechnical control) that minimize the selection pressure on pests.

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