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Residue Assessment of Bifenazate, Spirodiclofen and Abamectin in Strawberry Fruits Under Field Conditions

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Abstract: The dissipation behavior of three pesticides, bifenazate, spirodiclofen and abamectin was studied in strawberry fruits using a modified QuEChERS extraction technique. The residues of the tested acaricides were determined under field conditions after 1 hour (zero days), 1, 3, 7, 14, and 21 days. The maximum residue limits (MRLs) were 2, 2, and 0.15 mg/kg for bifenazate, spirodiclofen and abamectin, respectively. The strawberry fruits could be used safely after 15 days from bifenazate spraying and after 3 days from spirodiclofen spraying. The concentration of abamectin after 21 days did not reach MRL. The half-life values ($t_{1/2}$) of bifenazate, spirodiclofen, and abamectin in strawberry fruits were 0.99, 0.86, and 5.7 days, respectively.

1 Introduction

Pesticide residues in different foods whether fresh or processed are considered the most dangerous factors that threaten human health. To protect the environment and human health from pesticide risks, some organizations like the Codex Alimentarius Commission have fixed the maximum residue levels (MRLs) for each active ingredient in every crop. When a pesticide is used according to specified agricultural practices, the MRLs are the highest residue amounts in the food. To protect consumers' health, some government bodies and international organizations have set MRLs in food. Because laws governing MRLs have recently become increasingly detrimental, the European Union has established new recommendations for pesticides at low levels in vegetables to address these health issues (Mahmoud et al 2014).

For the separation and quantification of pesticide residues in vegetables and fruits, the chromatographic technique of high-performance liquid chromatography (HPLC) has been frequently used.

In Egypt, to control two-spotted spider mites, *Tetranychus urticae* (Acari: Tetranychidae), on vegetable and fruit plants, different insecticides, including bifenazate, spirodiclofen, and abamectin, have been used (ONSSA Commission Des Pesticides à Usage Agricole 2021). Bifenazate is a neural inhibitor and a nonsystemic acaricide with a contact-based mechanism of action and a lengthy residual effect (Pridgeon et al 2008). It has been used on citrus, tree fruits, nuts, and vegetables for phytophagous mites (eggs and motile stages). It was a new chemical group's acaricide (Hydrazine Carboxylates). Bifenazate is effective for controlling spider mites at all stages, from egg to adult. It affects the neurological system of the muscles. The mites became hyperactive and stopped feeding after around 3 hours and then died after 3-4 days (Tang et al 2014).

Spirodiclofen is an acaricide that kills mite eggs, nymphs, and adult females when they come in contact with it (adult males are unaffected). It functions as a lipid biosynthesis inhibitor and regulates mites in strawberry, citrus, grapefruit, lemon, lime, citron, and stone fruits related to ketoenol or tetronic acid chemical groups (Flores-Bernedo and Vásquez-Castro 2020).

Abamectin is a chemical made up of 80% avermectin B1a and 20% avermectin B1b. It is a member of the avermectin family of macrocyclic lactones manufactured by the soil actinomycete, *Streptomyces avermitilis.* It is a broad-spectrum acaricide with insecticidal qualities against few insects. It works by increasing the permeability of insect membranes to chloride ions, and it primarily increases the release of gamma-aminobutyric acid (GABA) (Batiha et al 2020).

Strawberry plants, *Fragaria* × *ananassa* Duchesne (Family: Rosaceae) are the most important crops of high economic importance as they occupy the forefront of export worldwide. Strawberries are grown commercially in 76 countries worldwide. China is the largest producer, with the United States, Mexico, Turkey, and Spain rounding out the top five. Production continues to increase, especially in Asia, North and Central America, and North Africa with a matching increase in demand in several parts of the world (Simpson 2018). Phytochemicals and vitamins abound in strawberries. In addition, they contain many polyphenols and antioxidants (Basu et al 2014).

This study is important in terms of consumer protection via determining the interval (PHI) of strawberry fruits sprayed with the three pesticides bifenazate, spirodiclofen, and abamectin under field conditions.

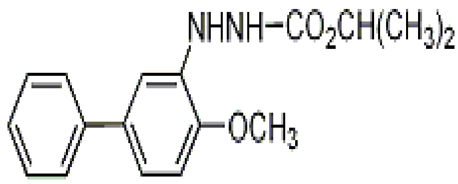
2 Materials and Methods

2.1 Pesticides selected for this study

Three acaricides are used extensively in Egypt for controlling two-spotted spider mites in strawberry plants and other Egyptian cultivations. Rates of acaricide application were chosen based on recommended rates on various crops in their soluble concentrate (Solo 24% SC and Concor 24% SC) and emulsifiable concentrate (Biomectin 5% EC) formulations were selected throughout the study (from Starchem Industrial Chemicals Company, Cairo, Egypt).

2.1.1 Bifenazate acaricides

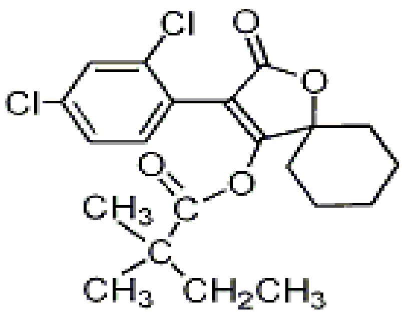
Chemical structure



Mode of action: Nonsystemic neuronal inhibitor with contact and residual activity. Inhibitor of electron transport in mitochondrial complex III.

2.1.2 Spirodiclofen acaricides

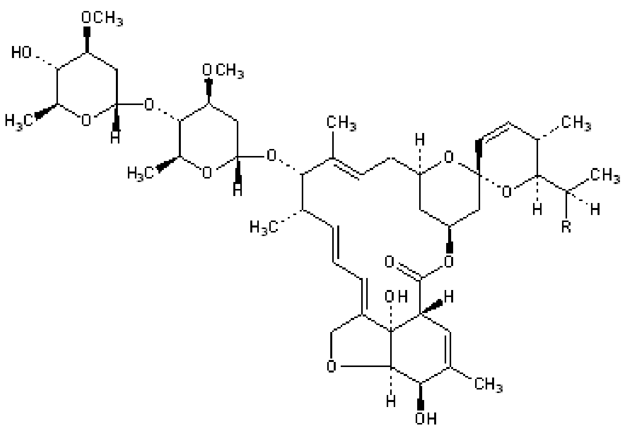
Chemical structure



Mode of action: Lipid synthesis inhibition

2.1.3 Abamectin acaricides

Chemical structure



component B_{1a}, R = C_2H_5 component B_{1b}, R = CH_3

Mode of action: Nerve poisons are avermectins. They activate the GABA system, a chemical "transmitter" manufactured at nerve endings that prevents communication between nerves and muscles.

2.2 Experimental design

2.2.1 Field study and sampling

This study was conducted in Elatta, Giza Governorate, Egypt, during the 2019 season at a private strawberry farm. Three insecticides were sprayed in a randomized complete block design with three replicates for each pesticide, (each replicate 20 m^2). The target area was sprayed with bifenazate, spirodiclofen, and abamectin at the recommended application rates of 75 cm^3, 30 cm^3 and 20 cm^3/100 L, respectively using a motor sprayer of 20-L capacity. Samples of strawberry fruits were randomly obtained from each replicate after 1 hour and 1, 3, 7, 14, and 21 days of application. Samples were taken to the laboratory in an ice box and kept in a freezer until pesticide residue analysis.

2.2.2 Pesticide residue analysis

The tested acaricides were analyzed in the Quality Control Laboratory in Starchem Industrial Chemicals Company, Cairo, Egypt.

2.2.3 Extraction and clean up

Bifenazate, spirodiclofen, and abamectin residues were obtained and cleaned using a modified QuEChERS technique (Lehotay et al 2005).

2.2.4 Determination of tested active ingredients on strawberry

Bifenazate

-The standard sample was prepared twice with 2.2 mg a.i in 2 mL acetonitrile and 2.4 mg a.i in 1 mL acetonitrile with a purity of 97%.
-The samples were analyzed using an HPLC instrument (Thermo Scientific) with a PDA Plus Detector (DAD) and a BDS HypersilC18 column with Dim (4.6 × 250 mm) and 5-µ particle size.
-The standard sample was injected with different volumes (0.5, 1, 2, 4, and 8 µL) from 2.2 mg and (0.25, 0.5, 1, 2, and 3 µL) from 2.4 mg to draw the calibration curve, while the unknown samples (0.5 mL) were injected with 10 µL.
-The mobile phase of bifenazate comprised acetonitrile: water (80:20), the minimum wavelength was 220 nm, the maximum wavelength was 360 nm, the flow rate was 1.5 ml/min, and the retention time was 7.94 min.

Spirodiclofen

-The standard sample was prepared using 2.4 mg a.i (purity of 99.1%) in 1 mL methanol.
-The samples were analyzed using an HPLC instrument (Shimadzu LC 2030) with a PDA Detector and a BDS HypersilC18 column with Dim (4. 6 × 250 mm) and 5-µ particle size.
-The standard sample was injected with different volumes (0.5, 1, 2, 4, and 8 µL) to draw the calibration curve, while the unknown samples (0.5 mL) were injected with 2 µL.
-The mobile phase of spirodiclofen was 100% methanol, the wavelength was 254 nm, the flow rate was 1 mL/min, and the retention time was 3.64 min.

Abamectin

The standard sample was prepared thrice with 2, 2, and 2.8 mg a.i (95% purity) in 1 mL methanol.
-The samples were analyzed using an HPLC instrument (Thermo Scientific) with a PDA plus Detector (DAD) and a BDS HypersilC18 column with Dim (4. 6 × 250mm) and a particle size of 5-µ.
-The standard sample was injected with different volumes (0.5, 1, 2, 4, and 8 µL) to draw the calibration curve, while the unknown samples (0.5 mL) were injected with 10 µL.
-The mobile phase of abamectin was methanol: water (90:10), the wavelength was 254 nm, the flow rate was 2 mL/min, and the retention time was 3.64 min.

2.2.5 Standards and recovery experiment

Bifenazate, spirodiclofen and abamectin were injected into three replicates of untreated strawberry samples (control) at 50, 100, and 50l g/kg, respectively. The samples were assessed according to the manufacturer's instructions, and the mean values of the three replicates were calculated. The recovery rates for the three pesticides were satisfactory, ranging from 82.7-89.5%. Bifenazate, spirodiclofen, and abamectin had minimal detection limits of 0.005, 0.001 and 0.003 mg/kg, respectively.

2.2.6 Statistical analysis

All data collected were statistically assessed and graphically depicted following the study of Timme and Fisher (1980), as cited in Thorbek and Hyder (2006). The following equations were used to calculate the half-lives ($t_{1/2}$) and tenth-lives ($t_{1/10}$) (Moye et al 1987):

$\mathbf{t_{(1/2)}}$ = Ln2/k

$\mathbf{t_{(1/10)}}$ = Ln10/k

$\mathbf{k_0}$ = ($1/t_x$). Ln a/bx.

$\mathbf{k_0}$ is the constant for the rate of degradation at hourly intervals.

k is the mean of k_0.

a is the initial residue level (zero time).

bx is the residual level at each of the hour's intervals.

3 Results and Discussion

3.1 Residues of selected acaricides on strawberry fruits

3.1.1 Bifenazate

Residues and dissipation percentages of bifenazate in strawberry fruits are shown in **Table 1** and **Fig 1.** The initial residue deposit, which remained on strawberry fruits 1 hour after treatment (zero days) was 37.7 ppm degrading to 18.5, 10.6, and 4.9 ppm at 1, 3, and 7 days, respectively, which was still above the MRL. at 14 and 21 days the residues were 1.94 and 0.95 ppm which were below the MRL=2 ppm set by FAO-WHO Codex Alimentarius Commission Recommended. Bifenazate residues in strawberry fruits were found to be below MRL of 2 mg/kg, showing that the strawberries could be used safely 15 days after being sprayed with bifenazate. Bifenazate had a half-life of 0.99 days in strawberry fruits. These findings contradict those of Mahmoud et al (2014), who found that the initial deposit of bifenazate was 5.89 $mgKg^{-1}$. The half-life $t_{1/2}$ of bifenazate was 2.31 days. Bifenazate residues were lost with PHI of 5 days, because the recommended dose they used was (35 cm^3/100 L water), half the dose that was used, and the spray was done in January, whereas the temperature was low. In China, Jian-hong et al (2014) reported that the half-life of bifenazate in strawberries was 3.15 days and the appropriate postharvest interval was 3 days. Moreover, Liu et al (2019) in China found that the half-life was 5.58 days while the PHI in strawberries was 3 days.

3.1.2 Spirodiclofen

Residues and dissipation percentages of spirodiclofen in strawberry fruits are indicated in **Table 1** and **Fig 2.** The initial residue deposit, which remained on strawberry fruits 1 hour after treatment (zero days), was 15.3 ppm, which degraded to 5.34 ppm with 65.1% loss; at 3 days, the deposit was 1.3 ppm with 19.5% loss, which was below the MRL of 2 ppm set by FAO-WHO. At 7, 14, and 21 days, the residues were nondetectable.

The spirodiclofen residues found in strawberry fruits were within the MRL of 2 kg**,** showing that the strawberries could be safely used after 3 days of being sprayed with spirodiclofen. These results are consistent with those of Morsy and El Hefni (2017) and Wang et al (2018) in strawberry fruits, and the half-life of spirodiclofen was 0.86 days.

3.1.3 Abamectin

Residues and degradation percentages of abamectin in strawberry fruits are shown in **Table 1** and **Fig 3.** The initial residue deposit, which remained on strawberry fruits 1 hour after treatment (zero days) was 3.82 ppm degrading to 2.78, 2.58, 1.41, 0.468, and 0.38 ppm after 1, 3, 7, 14, and 21 days of application, respectively. The concentration of abamectin after 21 days did not reach the MRL (0.15 mg/kg) according to the Codex Alimentarius Commission Recommended.

These findings contradict those of Mahmoud et al (2014) in Egypt, who stated that the half-life of abamectin was calculated to be 1.02 days. For the strawberries treated with abamectin, the PHI was 7 days. Abd El-Hamid et al (2015) in Egypt showed that the initial deposit of abamectin in strawberries was 1.31 mg/kg and the calculated half-life was 1.5 days. However, the PHI on strawberries was 7 days.

Table 1. Residues of bifenazate, spirodiclofen and abamectin acaricide in strawberry fruits

	Bifenazate				Spirodiclofen				Abamectin			
Days after treatment	**Residues µg/kg**	**Log. conc**	**% Dissipation**	**% Persistence**	**Residues µg/kg**	**Log. conc**	**% Dissipation**	**% Persistence**	**Residues µg/kg**	**Log. conc**	**% Dissipation**	**% Persistence**
Initial*	37.7	1.58	0.00	100	15.3	1.18	0.00	100	4.1	0.61	0.00	100
1	18.5	1.27	50.9	49.1	5.34	0.73	65.1	34.9	3.24	0.51	20.9	79.1
3	10.6	1.02	71.9	28.1	1.3	0.11	91.5	8.5	2.87	0.46	30	70
7	4.9	0.69	87	13	ND	ND	100	0.00	1.65	0.22	59.7	40.3
14	1.9	0.28	94.9	5.1	ND	ND	100	0.00	0.47	-0.33	88.5	11.5
21	0.95	-0.02	97.5	2.5	ND	ND	100	0.00	0.38	-0.42	90.7	9.3
K	**0.163**				**0.81**				**0.12**			
PHI	**14.9 days**				**2.4 days**				**26.6 days**			
MRL	**2mg/kg**				**2 mg/kg**				**0.15 mg/kg**			
$t_{1/2}$	**0.99 days**				**0.86 days**				**ln2/K= 5.7 days**			

* Samples were obtained after 1 hour of application

PHI = Preharvest interval

MRL = Maximum residue limit (Codex 2007 for bifenazate, Codex 2010 for spirodiclofen, and Codex 2016 for abamectin)

$t_{1/2}$ (days) = Residue half - life.

ND = Nondetectable

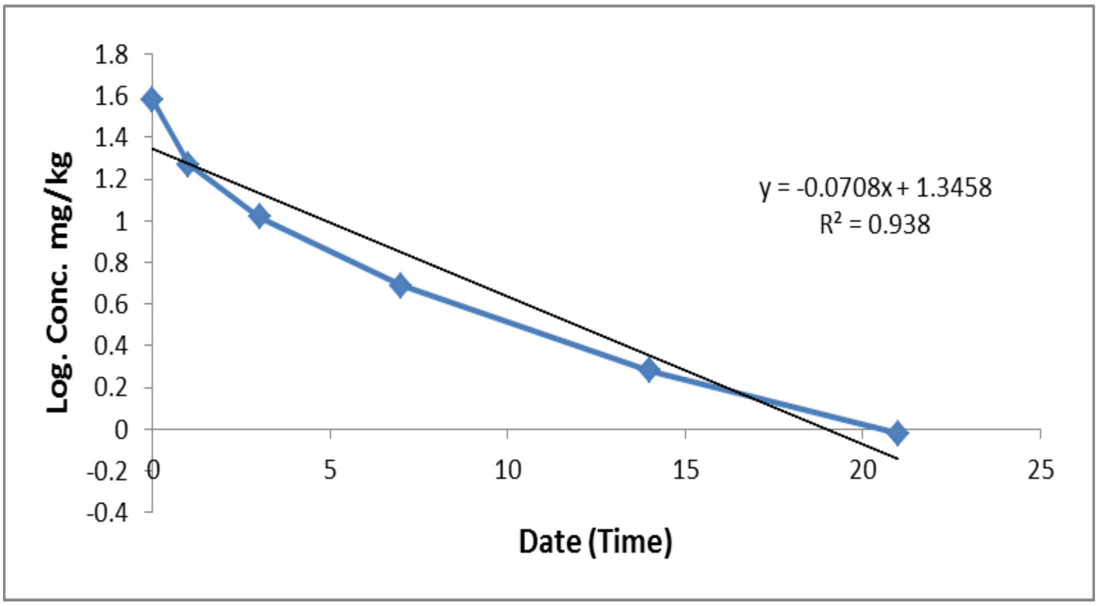


Fig 1. Log. Residue-day regression lines of bifenazate in strawberry fruits

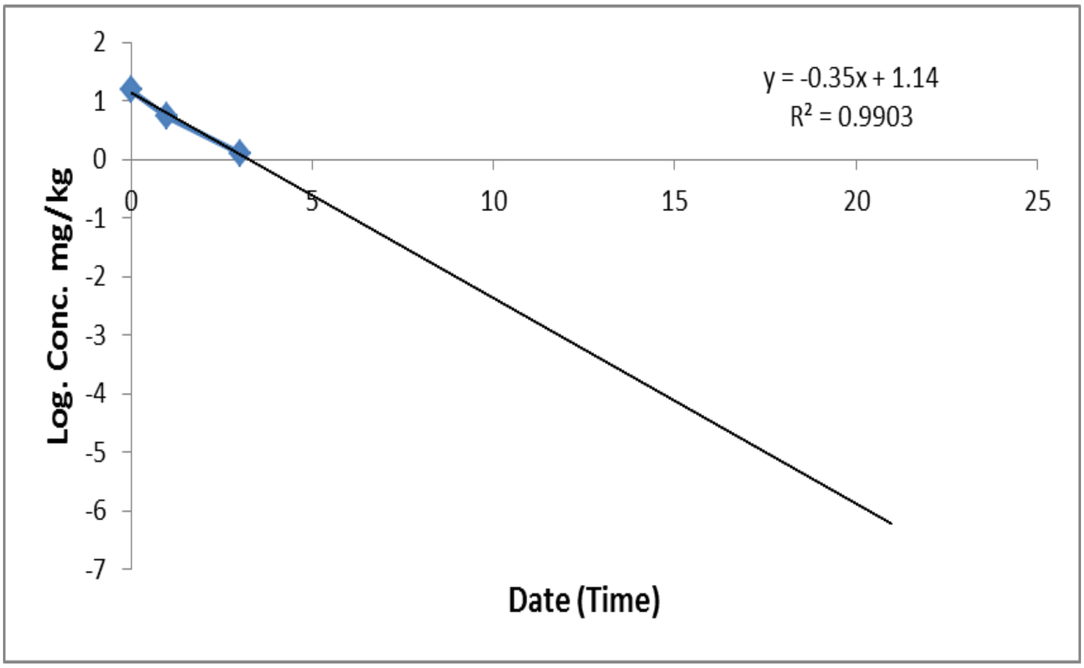


Fig 2. Log. Residue-day regression lines of spirodiclofen in strawberry fruits

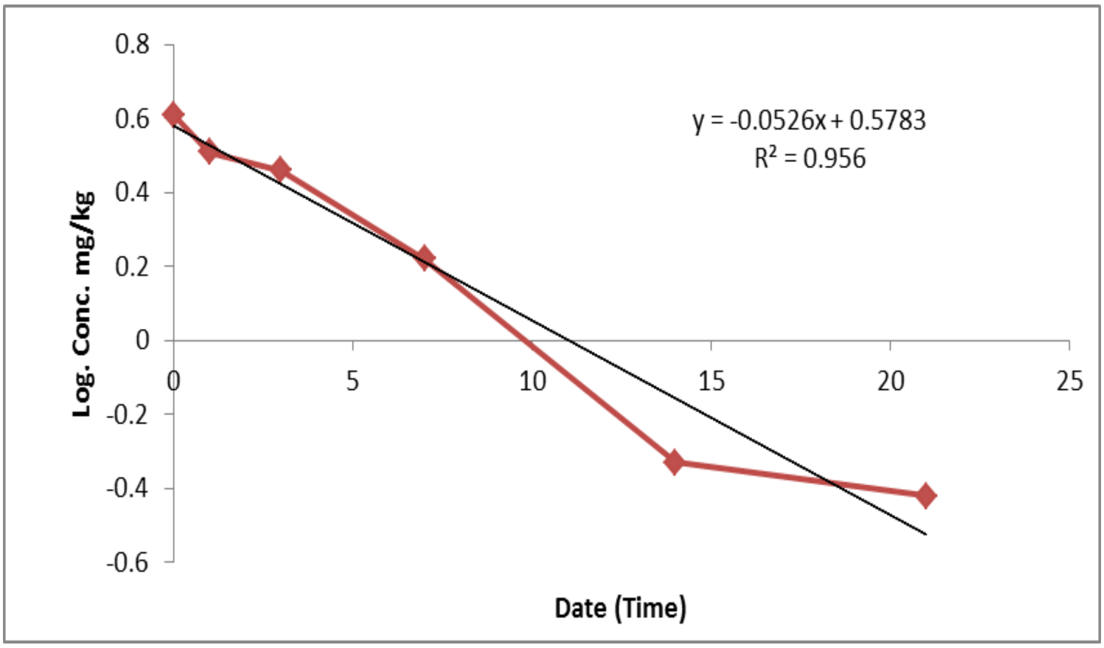


Fig 3. Log. Residue-day regression lines of abamectin in strawberry fruits

4 Conclusion

The dissipation of spirodiclofen (PHI = 3 days) and bifenazate (PHI = 14 days), was rapid, whereas the dissipation was more stable for abamectin (PHI < 21 days). The half-lives of spirodiclofen, bifenazate, and abamectin in strawberries fruits were 0.99, 0.86, and 5.7 days, respectively, based on application rate.

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