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# DETERMINATION OF SOME PESTICIDES RESIDUES IN FRUITS AND LEAVES OF GRAPE UNDER FIELD CONDITIONS BY HPLC

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## ABSTRACT

Hexythiazox, diniconazole, difenoconazole, spinetoram and methoxyfenozide pesticides were applied on the fruits and leaves of grapes grown in open field conditions. Their residues levels in fruits and leaves of grapes were detected at different times from initial times, one, three, seven and ten days after treatments. The rates of pesticide degradation, half-life value (t1/2) and pre-harvest interval (PHI) were also estimated. Pesticides were extracted and cleaned-up from samples by the QuEChERS method and then analyzed by HPLC-DAD. The estimated t1/2 values were 2.4 and 4.4 days, 0.9 and 1.6 days, 5.4 and 2.9 days, 1.09 and 1.07 days and 3.3 and 8.5 days for hexythiazox, diniconazole, difenoconazole, spinetoram and methoxyfenozide in fruits and leaves of grapes, respectively. Pre-harvest interval (PHI) values were 10 days and 15 days after application of diniconazole , hexythiazox and spinetoram to fruits and leaves of the grape .while pre-harvest interval (PHI) values were 7 days and 10 days after application of difenoconazole and methoxyfenozide to fruits and leaves of the grape .The maximum residue limits (MRL) were 0.5, 0.1, 3, 0.5 and 1 mg $kg^{-1}$ for hexythiazole, diniconazole, difenoconazole, spinetoram and methoxyfenozide, respectively. The fast degradation of hexythiazox, diniconazole, difenoconazole, and methoxyfenozide residues confirmed that they could be used as safe pesticides to reduce the health risks associated with the consumption of treated grapes.

**KEYWORDS:**
Residues, Grape, PHI, $t_{1/2.}$ HPLC, Pesticides, QuEChERS

## INTRODUCTION

The grape vine (*Vitis vinifera*) is a flowering plant native to the eastern Mediterranean region, central Europe, and southwestern Asia, starting from Morocco and Portugal to southern Germany and northern Iran [1]. In Egypt, grapes are grown from Aswan in the south to Alexandria in the north. In addition, many studies have found conclusive evidence that the consumption of fresh grapes can decrease the risk of cancer and cardiovascular diseases [2]and[3] Vieira et al. 2016) due to the presence of beneficial contents such as minerals, natural fiber, vitamins, and phytochemical compounds like flavonoids and anthocyanins [4]and[5] .

Hexythiazox is a non-systemic acaridae without toxic effects on mammals and helpful insects or mite predators. Hexythiazox is a key component in many integrated pest management programs and can be extensively used to control mites on cotton, fruits, and vegetables at any stage of plant growth from budding to fruiting [6] and [7]. Concerning the public health, the existence of hexythiazox residues have gained traction in recent years. The extraction and determination stages for hexythiazox have previously studied, as well as the role of the infrastructure of apparatus and facilities [8] and [9]. However, there is scarcity information on the destiny of hexythiazox under field conditions [6] and[10], and there are no conclusive researches evaluated the presence of hexythiazox residues in food commodities.

Diniconazole (DN) [a triazole fungicide] is one of the most important and widely used fungicides and it is directly worked though blocking the development of sterols, which are the major components of fungal cell membranes [11] and [12] . In grape, banana, cereal, and vine crops, diniconazole is commonly used to control ascomycetes and basidiomycetes [13]. The pesticide residue levels are estimated by maximum residue limits (MRLs), which are fixed for every region [14]. Difenoconazole (DFZ) is a triazole fungicide that has been found in estuaries and embayments in the aquatic environment[15]. DFZ is a systemic sterol demethylation inhibitor for the fungal enzyme sterol-1-4-a-demethylase, which is particularly efficient against illnesses causes by various fungi infecting cereals [16]. Moreover, it has been widely utilized in a variety of crops in several countries due to its capacity to combat numerous fungal infections.

Spinetoram/methoxyfenozide is a lepidopteran

insecticide to control lepidopteran pests in rice[17]. Spinetoram is a novel semi-syntetic insecticide and a multicomponent tetracyclic macrolide from the chemical group of spinosyns. It is a chemical modified product for the soil actinomycete, Saccharopolyspora spinose [18]. It is contained two closely related components, XDE-175-J and XDE-175-L at a ratio of approximately 3: 1 [19] and [20] . Spinosyns acts as allosteric modulators of nicotine acetylcholine receptors (nAChR)[21], and spinetoram is effective in the control of Lepidoptera, Thysanoptera and Coleoptera [22]. Methoxyfenozide is an extensively pesticide in agriculture to control pests and has no harmful effects on beneficial insect populations [23]. It is approved for usage in more than 50 countries and has increased to 15-fold between 2001 and 2015 [24].

Residues of pesticides lead to harmful impacts on the humans, especially if these commodities are freshly consumed. The intakes of any foodstuff that contain some pesticide residues can cause carcinogenic problems. So, it is preferable task to determine the residues of these pesticides and then evaluate the availability of treated agricultural products for human consumption.

## MATERIALS AND METHODS

**Chemical and reagents.** Methanol, ethyl acetate, methylene chloride, and acetone (Sigma-Aldrich, Steinheim, Germany) and the o-phosphoric acid (El-Nasr Company, Cairo, Egypt) were purchased to be used as reagents for the HPLC. The exact concentrations used to build the calibration graph were 0.01, 0,02, 0.05, 0.1 and 0.5 for hexythiazox and diniconazole, 0.02, 0,05, 0.1, 0.5 and 0.75 for difenoconazole, 0.2, 0.5, 2.5 and 5 for spinotram and 0.5, 1, 2.5 and 5 for methoxyfenozide.

**Pesticides standard solution.** 100 µg/ml in ethyl acetate for tested pesticides from Central Agricultural Pesticides Laboratory, Cairo, Egypt. It was mixed with the pesticides to prepare their standard solutions.

**Pesticide technical formulations.** Hexythiazox was purchased from Biostad, Cairo, Egypt, diniconazole was obtained from El-Quorma shop,Cairo, Egypt and difenoconazole was obtained from Damak shop, Cairo, Egypt. However, spinetoram and methoxyfenozide was obtained from DowAgroSciences-USA.

**Field experiment.** Experiment was carried out in five districts [four districts were set up for the pesticide treatments and the fifth area was for the control (without pesticides)] at El Qalubia Governorate, Egypt. Hexythiazox (Maiden 5.45% EC), diniconazole (Sumy-zed 5% EC), difenoconazole (canon12.5% SC) and Spinetoram/methoxyfenozide (uphold 36% SC) were applied in July 16, 2020 at the recommended dose [hexythiazox (400ml/100L), diniconazole (35ml/100L), difenoconazole (75ml/100L) and Spinetoram/methoxyfenozide (125ml/100 L) using a knapsack sprayer fitted with a single nozzle.

**Sampling.** The samples of fruits and leaves of grapes were randomly packed up at one hour, 1, 3, 7, 10 and 15 days after application of the above-mentioned pesticides. The collected samples of fruits and grape leaves (two-three kg) were transferred to the laboratory in an ice box, homogenized, divided into subsamples (50 g for fruits and 25 g for leaves), and finally stored at -20 °C in a deep freezer for further analyses of pesticide residues.

**Extraction and clean-up.** The used pesticides were extracted using acetonitrile and then separating after adding a salt combination by QuEChERS method [25] and [26] .

**TABLE 1**
**HPLC conditions for detecting of Hexythiazox, Diniconazole, Difenconazole, Spinotramand Methoxyfenozide, in addition to their retention times.**

| Pesticides | Detection wavelength | Flow rate | Mobile phase | Retention time |
|---|---|---|---|---|
| Hexythiazox | 250 | 0.8 | acetonitrile: water (70:30 v/v) | 2.417 |
| Diniconazole | 210 | 1 | acetonitrile : water (60:40 v/v) | 3.355 |
| Difenconazole | 230 | 1 | Methanol : water (95: 5v/v) | 5.093 |
| Spinotram | 245 | 0.8 | acetonitrile: water (50:50 v/v) | 5.445 |
| Methoxyfenozide | 210 | 0.8 | acetonitrile: water (80:20 v/v) | 3.566 |

**Recovery of residues.** Control samples of fruits and leaves of grape were spiked with a known amount of hexythiazox , diniconazole, difenoconazole and Spinetoram/methoxyfenozide before the extraction and clean -up for recovery tests of the pesticides. Samples were passed through the entire process of extraction, cleaned up and analyzed. Percent of recovery was calculated by the following equation as:

% Recovery = (µg) present/ (µg) added) ×100.

The spiked levels were 0.5 and 1.0 mg/kg. These data were corrected according to the recovery rate.

**Analysis.** HPLC conditions for detecting of Hexythiazox, Diniconazole, Difenconazole, Spinotramand Methoxyfenozide, in addition to their retention times were presented in Table 1.

**Hexythiazox.** High performance Liquid Chromatography (HPLC-DAD) Agilent 1260 series was used to determine the values of hexythiazox. The analytical column Nucleosil-C18, 5um (4 X 250 mm) and a UV diode array detector set at 250 nm was utilized at a flow rate of 0.8ml/min, the mobile phase was acetonitrile: water (70:30 v/v). Hexythiazox (one peak) had a retention time (Rt) of 2.417 minutes.

**Diniconazole.** The analysis was conducted with an Agilent 1260 HPLC equipped with a diode array detector (Agilent, Palo-Alto, CA, USA). A reverse-phase C18 HPLC hypersil column (4 mm (i.d) 150 mm length) was used as the separation column and was maintained at 25°C. With a flow rate of 1 ml/min, the mobile phase was made up of 60:40 acetonitrile and water. The UV light had a wavelength of 210 nm. Diniconazole (one peak) had retention time of 3.355 minutes.

**Difenoconazole.** HPLC was performed using an Agilent 1260 series liquid chromatography system with a diode array detector (DAD) [Agilent, Palo-Alto, CA, USA]. A wavelength UV-vis detector and a reverse-phase C-18 column (150 mm × 4.6 mm × 5 µm) were used at a flow rate of 1.0 mL·min$^{-1}$. A mobile phase of methanol and water (v/v = 95: 5 for difenoconazole) was used for the isocratic elution condition. The detection wavelength for difenoconazole was 230 nm.A retention time (Rt) of 5.093 minutes.

**Spinetoram.** High performance Liquid Chromatography (HPLC) Agilent 1260 series was used to determine the values of spinetoram. The analytical column Nucleosil-C18, 5um (4 X 250 mm) and a diode array detector (Agilent, Palo-Alto, CA, USA) set at 245nm was utilized at a flow rate of 0.8 ml/min, the mobile phase was acetonitrile: water (50:50 v/v). Spinetoram (one peak) had a retention time (Rt) of 5.445 minutes.

**Methoxyfenozid.** (HPLC) Agilent 1260 series was used to determine the values of spinetoram. The analytical column Nucleosil-C18, 5um (4 X 250 mm) and a UV diode array detector set at 210nm was utilized at a flow rate of 0.8 ml/min, the mobile phase was acetonitrile: water (80:20 v/v).

**Calculation of the residues and half-life (t1/2).** The residues were computed using the equation of Möllhoff [27] The half-life time (t1/2) of each pesticide studied was computed using the equation of [28].

## RESULTS AND DISCUSSION

**Method validation.** The residues of hexythiazox, diniconazole, difenoconazole and Spinetoram/methoxyfenozide in fruits and leaves of grape samples were detected using HPLC. The peak regions of the samples were compared to external standards produced from unfortified extracts to determine the recovery. The method specificity demonstrated that there was no interference from the solvent or matrix. A standard calibration curve prepared with an ethyl acetate stock solution was used for quantification. Good linearity was achieved between 0.1 to 0.5 g/ml with a correlation of 0.99871, 0.99766, 99944, 99989 and 99698 for hexythiazole, diniconazole, difenoconazolem, spinetoram and methoxyfenozide, respectively .

The fruit and grape leaves samples were tested at 0.1 mg/kg spiked level with six replicates. The methodologies for extracting and analyzing hexythiazox, diniconazole difenoconazole, spinetoram and methoxyfenozide residues in grape leaves and fruit samples were confirmed by their mean recoveries at different fortification levels. Data in Table 2 indicated that the mean recovery values of hexythiazox were 89% and 84.3% for fruits and leaves of grape. In the case of diniconazole, these values were 100% and 87.1% for fruit and leaves of grape. In difenoconazole were 86.5% and 90% for fruit and leaves of grape, while were 88.15% and 85.65% in Spinetoram. In methoxyfenozide, these values were 91.5% and 90% for fruit and leaves of grape, respectively. LODs were 0.1, 0.01, 0.1, 0.01 and 0.01 µg/kg while LOQs were 0.3, 0.01, 0.1, 0.03and 0.1 µg/kg for hexythiazox ,diniconazole, difenoconazolem, spinetoram and methoxyfenozide, respectively.

**TABLE 2**
**Recoveries and parameters of hexythiazox, diniconazole, difenoconazolem, spinetoram and methoxyfenozide.**

| Pesticides | Samples | Insecticide level (ppm) | Recovery% ± RSD% | Average (%) | $LOD_{s\ (\mu g/kg)}$ | LOQs (ppm) |
|---|---|---|---|---|---|---|
| Hexythiazox | Fruits | 1 | 88.0±0.08 | 89 | 0.1 | 0.3 |
| | | 0.5 | 90.0±0.01 | | | |
| | Leaves | 1 | 85.7±0.025 | 84.3 | | |
| | | 0.5 | 83.0±0.022 | | | |
| Diniconazole | Fruits | 1 | 99.0±0.012 | 100 | 0.01 | 0.01 |
| | | 0.5 | 101.0±0.018 | | | |
| | Leaves | 1 | 88.3±0.42 | 87.1 | | |
| | | 0.5 | 86.0±0.018 | | | |
| Difenoconazole | Fruits | 1 | 90.0±0.21 | 86.5 | 0.1 | 0.1 |
| | | 0.5 | 83.0±0.012 | | | |
| | Leaves | 1 | 94.0±0.11 | 90 .0 | | |
| | | 0.5 | 86.0±0.02 | | | |
| Spinetoram | Fruits | 1 | 87.3±0.02 | 88.15 | 0.01 | 0.03 |
| | | 0.5 | 89.0±0.011 | | | |
| | Leaves | 1 | 85.3±0.32 | 85.65 | | |
| | | 0.5 | 86.0±0.61 | | | |
| Methoxyfenozide | Fruits | 1 | 93.0±0. 12 | 91.5 | 0.01 | 0.1 |
| | | 0.5 | 90.0±0.06 | | | |
| | Leaves | 1 | 89.0±0.41 | 90.0 | | |
| | | 0.5 | 91.0±0.30 | | | |

**Hexythiazox residues.** Results in Table 3 showed the hexythiazox residues in fruits and leaves of grapes. One hour after applications, hexythiazox residues were 1.28 and 0.98 mg/kg, respectively. Fruits and leaves of grapes contained 0.96 and 0.81 mg/kg with a loss of 25.00% and 17.34% of the initial amounts of hexythiazox for fruits and leaves, respectively after one day. Concentrations of Hexythiazox residues in fruits of grape were 0.96, 0.55, 0.12, 0 mg/kg, while in the leaves were 0.81 to 0.69, 0.50, 0 mg/kg after 1, 3, 7 and 10 days, respectively. The losses of hexythiazox were gradually increased after 1, 3 and 7 days from its application to be 25.00, 57.03 and 92.18%, respectively for fruits and 17.34, 29.59 and 48.98%, respectively for leaves. Hexythiazox had half-lives of 2.4 and 4.4 days in grape fruits and leaves, respectively. The final residues levels of hexythiazox were 0.12 and 0.50 mg/kg for fruits and leaves of grape after 7 days. According to the EU [29], the MRLs for hexythiazox residues on fruit grapes were 0.05 mg/kg. These data indicated that grape fruits might be used safely one week after spraying hexythiazox.

These findings are consistent with those of [6 ] , who observed an initial deposit of 0.76 mg/kg in bean pods after using hexythiazox at the indicated dosage. While, [ 30 ] found after thin-layer sun exposure that the half-life duration of hexythiazox estimated using pseudo-first-order kinetics was larger than 8 days, indicating the likelihood of residues surviving in food. Furthermore,[7] found that hexythiazox was dispersed in strawberries, with half-lives ranging from 3.43 to 3.81 days. The residue of hexythiazox in strawberries decreased from 0.782 to 0.04 mg/kg, well below the Codex MRL of 6 mg/kg for strawberries. [31 ] indicated that the residues of hexythiazox were safe on the 5th and 7th day after spraying. The half-life of hexythiazox ranged from 1.10 to 1.82 days and the safe waiting period of 3.8 days might be recommended for harvesting the tea leaves after spraying hexythiazox. As explained [32], the half-life value ranged between 1.43-2.01 days of hexythiazox in okra; The Pre harvest interval of Hexythiazox in okra was calculated and found in the range of 2-5 days.

**Diniconazole residues.** The persistence of diniconazole in grapes under open-field conditions was presented in Table 4 Results showed that residues in fruits and leaves of grape diniconazole (initial deposit) were 1.29 and 3.49 mg/kg, respectively. The amounts of diniconazole in the fruits treated decreased to 0.58 and 0.02 mg/kg after 1 and 3 days of application. The degradation of diniconazole in the grape leaves were 2.42, 0.47 and 0.03 mg/kg after 1, 3 and 7 days after treatments, respectively. After 7 and 10 days of treatments, they were no detectable amounts for the diniconazole in the fruits but in the leaves, no detectable values for diniconazole after 10 days. The losses of diniconazole increased gradually after 1 and 3 days from the application to be 55.03 and 99.14%, respectively for fruits and 30.65, 86.53 and 99.14%, respectively for leaves. The half-life values (t1/2) of diniconazole in fruits and leaves of

grapes were 0.9 and 1.6 days, respectively. According to [29], the value of MRLs for diniconazole residues in fruit grapes was 0.01 mg/kg, while PHI was 10 days for fruits and 15 days for leaves of grapes. Our findings are consistent with those of [33 ] who tested the residues of diniconazole (azole fungicide) on and in grape leaves and discovered that the safety period (that should be waited) before marketing grape leaves is at least three weeks. The fungicide propiconazole residues in wheat straw and leaves were studied for two years. At first, the fungicide deposits on straw and leaves were around one-quarter of the total dose applied to all treatment plots . Moreover, the fungal residues vanished quickly, and the fungicide's half-life in straw and leaves was about 5 days. Fungicide residues in grain were low at harvest time, but in straw and leaves were high, particularly at the greater spraying rates compared with the permitted dosage. [34] recorded that penconazole (a triazole fungicide) residues decreased in grapes with increasing the time. The residues of penconazole were dissipated to an extent of 14.42% after 1 day, and by 73.08% after 10 days after spraying. After 14 days of treatment, the residue of penconazole in grapes was below 0.02 mg/kg. Also, they found that the penconazole half-life value (t1/2) on grapes was 1.56 days at the recommended dosage. [35] observed the residues of penconazole, which used as a common pesticide for the production of cucumber under the greenhouse conditions. The maximum residual limits (MRLs) for penconazole in cucumber were 0.06 mg/kg according to a Codex Alimentarius Commission statement. Penconazole had a half-life of 13.4 (8.4–14) days. Several elements, including light, heat, pH, and moisture, might influence the pesticide persistence, as well as the effect of some chemical and physical components [36]and [37].

**Difenoconazole residues.** The persistence of difenoconazole in grapes under open-field conditions was presented in Table 5. Results showed that residues in fruits and leaves of grape difenoconazole (initial deposit) were 0.92 and 5.84 mg/kg, respectively. The amounts of difenoconazole in the fruits treated decreased to 0.79, 0.66, 0.45 and 0.21 mg/kg after 1, 3, 7 and 10 days of application, respectively. The degradation of difenoconazole in the grape leaves were 4.65, 2.78, 2.62 and 2.20 mg/kg after 1, 3, 7 and 10 days after treatments, respectively. After 15 days of treatments, they were no detectable amounts for the difenoconazole in the fruits and leaves. The losses of difenoconazole increased gradually after 1, 3, 7and 10 days from the application to be 14.13, 28.26, 51.08 and 77.17%, respectively for fruits and 20.37, 52.39, 55.13 and 62.32%, respectively for leaves. The half-life values (t1/2) of difenoconazole in fruits and leaves of grapes were 5.4 and 2.9 days, respectively. According to [29], the value of MRLs for difenoconazole residues in fruit grapes was 3 mg/kg, while PHI was 17 and 21 days for fruits and leaves of grapes.

**TABLE 3**
**Residue levels of hexythiazox in fruits and grape leaves.**

| Time interval (days) | Fruits | | | Leaves | | |
|---|---|---|---|---|---|---|
| | Residues mg/kg | Loss (%) | Persistence (%) | Residues mg/kg | Loss (%) | Persistence (%) |
| Initial | 1.28± 0.005 | 0.00 | 100 | 0.98± 0.004 | 0.00 | 100 |
| 1 | 0.96± 0.011 | 25.00 | 75.00 | 0.81± 0.005 | 17.34 | 82.65 |
| 3 | 0.55± 0.007 | 57.03 | 42.96 | 0.69± 0.006 | 29.59 | 70.40 |
| 7 | 0.12± 0.004 | 92.18 | 7.82 | 0.50± 0.006 | 48.98 | 51.02 |
| 10 | ND | - | - | ND | - | - |
| T½ (days) | 2.4 | | | 4.4 | | |
| MRL | 0.05 mg/kg ( EU, 2013) | | | 0.05 mg/kg (EU, 2013) | | |
| PHI | 10 | | | 15 | | |

Initial: one hour after spraying. Data as mean ± SE. ND: Non Detected.

**TABLE 4**
**Residues level of diniconazole in/on fruits and leaves grape.**

| Time interval (days) | Fruits | | | Leaves | | |
|---|---|---|---|---|---|---|
| | Residues (ppm) | Loss (%) | persistence (%) | Residues (ppm) | Loss (%) | persistence (%) |
| Initial | 1.29± 0.001 | 0.00 | 100 | 3.49±0.035 | 0.00 | 100 |
| 1 | 0.58± 0.012 | 55.03 | 44.96 | 2.42±0.003 | 30.65 | 69.34 |
| 3 | 0.02± 0.004 | 99.44 | 1.55 | 0.47±0.012 | 86.53 | 13.46 |
| 7 | ND | - | - | 0.03±0.005 | 99.14 | 0.85 |
| 10 | ND | - | - | ND | - | - |
| T½ (days) | 0.9 | | | 1.6 | | |
| MRL | 0.01 mg/kg( EU, 2013) | | | 0.01 mg/kg( EU, 2013) | | |
| PHI | 10 | | | 15 | | |

Initial: one hour after spraying. Data as mean± SE. ND: Non Detectable.

**TABLE 5**
**Residue levels of difenoconazole in/on fruits and grape leaves.**

| Time interval (days) | Fruits | | | Leaves | | |
|---|---|---|---|---|---|---|
| | Residues mg/kg | Los s (%) | Persistence (%) | Residues mg/kg | Loss (%) | Persistence (%) |
| Initial | 0.92± 0.001 | 0.00 | 100 | 5.84±0.011 | 0.00 | 100 |
| 1 | 0.79± 0.032 | 14.13 | 85.86 | 4.65± 0.001 | 20.37 | 79.62 |
| 3 | 0.66± 0.011 | 28.26 | 71.73 | 2.78± 0.003 | 52.39 | 47.60 |
| 7 | 0.45± 0.003 | 51.08 | 48.91 | 2.62± 0.005 | 55.13 | 44.86 |
| 10 | 0.21± 0.003 | 77.17 | 22.82 | 2.20± 0.004 | 62.32 | 37.68 |
| 15 | ND | - | - | ND | - | - |
| T½ (days) | 5.4 | | | 2.9 | | |
| MRL | 3 mg/kg ( EU, 2013) | | | 3 mg/kg( EU, 2013) | | |
| PHI | 7 | | | 10 | | |

Initial: one hour after spraying. Data as mean± SE. ND: Non Detectable.

In previous studies, the residue concentrations of difenoconazole in sweet persimmons ranged from 0.20.56 mg/kg after 1 to 21 days after spraying, and the residue amount was reduced below the MRL level, 1.0 mg/kg, following 1 day harvest, according to [38] The residual quantities of difenoconazole in sweet persimmon did not surpass the MRL set limits. According to [39], the computed half-lives (to.5) for difenoconazole were 4.494 days in grape berries and 35.134 days in leaves following application. After harvesting the grape berries and leaves for difenconazole, a waiting period of at least 7 and 10 days is required.

**Spinetoram residues.** Results in Table 6 showed the spinetoram residues in fruits and leaves of grapes. One hour after application, spinetoram residues were 9.54 and 14.75 mg/kg, respectively. Fruits and leaves of grapes contained 5.13 and 7.86 mg/kg with a loss of 25.00% and 46.71% of the initial amounts of spinetoram for fruits and leaves, respectively after one day. Concentrations of spinetoram residues in fruits of grape were 2.36, 1.67 mg/kg, while in the leaves were 5.23 and 2.43 mg/kg after 3 and 7 days, respectively. The losses of spinetoram were gradually increased after 1, 3 and 7 days from its application to be 25.00,57.03 and 92.18%, respectively for fruits and 46.71,64.54 and 83.52%, respectively for leaves. Spinetoram had a half-life of 1.09 and 1.07 days in grape fruits and leaves, respectively. The final residues of spinetoram were 0.87 mg/kg for leaves of grape after 10 days. According to [29], the MRLs for spinetoram residues on fruit grapes were 0. 5 mg/kg. These data indicated that grape fruits might be used safely 10 days after spraying spinetoram.

Our findings are consistent with those of [40] who recorded that spinetoram residues in pear fruits were determined using QuEChERS method followed by HPLC-DAD. Three days after the application, spinetoram residues in pear fruits were below the MRL (0.2 mg/kg). The results have shown that spinetoram dissipation pattern with a half-life of 2.17 days, in pear fruits. [41] indicated that spinetoram was sprayed on tomato at recommended dose and tomato fruit samples were collected at zero time (one hour after application), 1, 3, 5, 7, 10 and 15 days after application. Recoveries were ranged between 88.81-95.41% with RSD of 3.4 -7.0% in tomato with fortification levels of 0.1, 0.5 and 1.0 mg/kg, respectively. Limit of quantification (LOQ) of this method

**TABLE 6**
**Residue levels of spinetoram in/on fruits and grape leaves.**

| Time interval (days) | Fruits | | | Leaves | | |
|---|---|---|---|---|---|---|
| | Residues mg/kg | Loss (%) | Persistence (%) | Residues mg/kg | Loss (%) | Persistence (%) |
| Initial | 9.45± 0.001 | 0.00 | 100 | 14.75±0.011 | 0.00 | 100 |
| 1 | 5.13± 0.032 | 25.00 | 75.00 | 7.86± 0.001 | 46.71 | 53.29 |
| 3 | 2.36± 0.011 | 57.03 | 42.96 | 5.23± 0.003 | 64.54 | 35.45 |
| 7 | 1.67± 0.003 | 92.18 | 7.82 | 2.43± 0.005 | 83.52 | 16.47 |
| 10 | ND | - | - | 0.87± 0.004 | 94.10 | 5.89 |
| 15 | ND | | | ND | - | - |
| T½ (days) | 1.09 | | | 1.07 | | |
| MRL | 0. 5 mg/kg ( EU, 2013) | | | 0. 5 mg/kg( EU, 2013) | | |
| PHI | 10 | | | 15 | | |

Initial: one hour after spraying. Data as mean± SE. ND: Non Detectable.

**TABLE 7**
**Residue levels of methoxyfenozide in/on fruits and grape leaves.**

| Time interval (days) | Fruits | | | Leaves | | |
|---|---|---|---|---|---|---|
| | Residues | Loss | Persistence | Residues | Loss | Persistence |
| | mg/kg | (%) | (%) | mg/kg | (%) | (%) |
| Initial | 0.69± 0.001 | 0.00 | 100 | 1.20±0.011 | 0.00 | 100 |
| 1 | 0.57± 0.032 | 17.39 | 82.60 | 1.06± 0.001 | 11.66 | 88.33 |
| 3 | 0.38± 0.011 | 44.92 | 55.07 | 0.96± 0.003 | 20 | 80 |
| 7 | 0.12± 0.003 | 55.89 | 44.11 | 0.71± 0.005 | 40.83 | 59.17 |
| 10 | ND | - | - | 0.20± 0.004 | 83.33 | 11.66 |
| 15 | ND | - | - | ND | - | - |
| T½ (days) | 3.3 | | | 8.5 | | |
| MRL | 1 mg/kg ( EU, 2013) | | | 1 mg/kg( EU, 2013) | | |
| PHI | 7 | | | 10 | | |

Initial: one hour after spraying. Data as mean± SE. ND: Non Detectable.

was found to be 0.1 mg/kg, while limit of detection was 0.005 mg/kg. Half-life (t½) and pre-harvest interval (PHI) were studied and they were 2.71 and 10 days respectively. [42] showed that spinetoram dissipated rapidly from 0.62 to 0.36 mg/kg with a loss percentage 41.9% in the first day after application in pepper, while in cabbage spinetoram dissipated from 0.33 to 0.12 mg/kg with a loss percentage 63.6% in the first day after application in a field trial.

**Methoxyfenozide residues.** The persistence of methoxyfenozide in grapes under open-field conditions was presented in Table 7. Results showed that residues in fruits and leaves of grape methoxyfenozide (initial deposit) were 0.69 and 1.20 mg/kg, respectively. The amounts of methoxyfenozide in the treated fruits decreased to 0.57 and 0.38 mg/kg after 1 and 3 days of application, and reached 0.12 mg/kg after 7 days. The degradation of methoxyfenozide in the grape leaves were 1.06, 0.96, 0.71 and 0.20 mg/kg after 1, 3, 7 and 10 days after treatments, respectively. After 10 and 15 days of treatments, they were no detectable amounts for the methoxyfenozide in the fruits but in the leaves, no detectable values for methoxyfenozide after 15 days. The losses of methoxyfenozide increased gradually after 1, 3 and 7 days from the application by 17.39, 44.92 and 55.89%, respectively for fruits while reached 11.66, 20, 40.83 and 83.33% after 1, 3, 7 and 10 days, respectively for leaves. The half-life values (t1/2) of methoxyfenozide in fruits and leaves of grapes were 3.3 and 8.5 days, respectively. According to [29], the value of MRLs for methoxyfenozide residues in fruit grapes was 1 mg/kg, so fruits and leaves of grapes could be used safely after 1 day from the spray with methoxyfenozide, while PHI was 7 days for fruits and 10 for leaves of grapes. Our findings were not consistent with those of [43], who showed that methoxyfenozide dissipated in cauliflower with the half-lives (t1/2) at 2.5-3.5 days and in tea with t1/2 at 1.2 days under field conditions in China.

## CONCLUSION

Our results concluded that, after recommended application of hexythiazox in fruits and leaves of grapes, the half-lives were 2.4 and 4.4 days, with safe period of 10 and 15 days respectively. Concerning, Diniconazole the calculated half-lives were 0.9 and 1.6 days accompanied with 10 and 15 days' safe period, respectively. Regarding, spraying difenoconazole in grapes under open-field conditions with recommended application dose, the half-lives were 5.4 and 2.9 days with safe period of 7 and 10 days in fruits and leaves, respectively. For spinetoram residues in grasp, the obtained half-lives were 1.09 and 1.07 with safe period of 10 and 15 days for fruits and leaves, respectively. Finally, methoxyfenozide showed half-lives 3.3 and 8.5 days with safe period 7 and 10 days, for fruits and leaves, respectively. In recommendation, leaves safely harvested after 15 days from studied pesticides application, while, fruits safely used after 10 days.

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