**Dissipation Behavior of Chlorfenapyr and Difenoconazole Residues in/on Grapes (*[Vitis vinifera](https://www.google.com.sa/search?q=Vitis+vinifera+L&spell=1&sa=X&ei=QaGQUsCHMpSThgewnYGwCA&ved=0CC4QvwUoAA)* [L](https://www.google.com.sa/search?q=Vitis+vinifera+L&spell=1&sa=X&ei=QaGQUsCHMpSThgewnYGwCA&ved=0CC4QvwUoAA).)**

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**Abstract:** Excessive use of pesticides in grape cultivation could lead to impact on environment and health. Dissipation rate, half-live (t0.5) and the preharvest interval (PHI) of acaridae chlorfenapyr (challenger 36% SC) and a fungicide difenoconazole (Score 25% EC) which two widely used pesticides in growing grape, were evaluated in grape fruits and leaves.. Samples were collected randomly at 1 h to 22 days after the pesticides application at recommended dose. Pesticides residues were quantified by using gas chromatography equipped with micro electron capture detector (GC-µECD) after extraction and clean up. Results showed that the initial deposits were 1.923 and 1.773mg kg-1 in grapes berries and 4.158 and3.642 mg kg-1 in leaves for chlorfenapyr and difenoconazole, respectively, the Chlorfenapyr and difenoconazole obey first order kinetics with dissipation rates 0.386, 0.294 days-1 and 0.154, 0.135 days-1in grape berries and leaves, respectively. The calculated half-life’s (to.5) were 1.796 and 4.494 days in grapes berries and 2.359 and 35.134 days in leaves after the application, respectively. We suggested that a waiting period of at least 15 and 17 days before harvesting the grape berries and leaves for chlorfenapyr, 17 and 21 days for difenconazole, respectively after the application at recommended dose that may be considered quite safe from point of health hazards due to toxic effect of residues.

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**1. Introduction**

White seedless table grape are one of the most widely grown fruit crops in the world. In Egypt**,** grapes considered to be the second most important fruit crops after citrus (**Anonymous, 2013**). Powdery mildew, spider mite, two spotted spider mites, thrips and aphids are of the most important fungal and insect pests of grape vines and leave all over the world, and causes significant economic damage in terms of yield and quality deterioration of grapes. (**Ellen *et al*., 1997;** **Banerjee *et al.,* 2008)**.

Chlorfenapyr [4-bromo-2-(4-chlorophenyl)-1-ethoxymethyl-5-trifluoromethyl-1*H*-pyrrole-3-carbonitrile] is a novel broad spectrum non systemic insecticide applied for the control of various insect and mite pests on cotton, ornamentals, and a number of vegetable crops. Chlorfenapyr is actually a pro-insecticide that is converted to an active metabolite in the midget of insects and mites **(Lovell *et al.,* 1990(.**

Difenoconazole [3-chloro-4-[(2*RS*,4*RS*;2*RS*,4*SR*)-4-methyl-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether] is a broad spectrum fungicide that controls a wide variety of fungi-including members of the Aschomycetes, Basidomycetes and Deuteromycetes families. It acts as a seed treatment, foliar spray and systemic fungicide. It is taken up through the surface of the infected plant and is translocated to all parts of the plant. It has a curative effect and a preventative effect**.(Ellen *et al*., 1997).**

Previous studies reported that the dissipation of chlorfenapyr in cabbage, Pakchoi, cucumber, soybean, grape, chilli were examined (**Cao *et al*., 2005; OU X.M. *et al*., 2006; yang-ling *et al*., 2007; Li-Ping 2008; Banerjee *al.*, 2008; Mukhopadhyay *et al*., 2012**). Also the dissipation behavior of difenoconazole in apple leaves, grass, apple orchard soils, Chinese cabbage and its growth soil, Tomatoes, Paddy field, cucumber were examined (**Jacob and Werner 1996; Ellen *et al.,* 1997; Wang *et al*., 2008, Nasr *et al*., 2009; Ruilan *et al*., 2010; Di, *et al*., 2010**). However, those studies were not comprehensive and representative; more studies were needed to evaluated environmental safety of chlorfenapyr and difenoconazole.

To our knowledge there is no data has published on the dissipation of these to pesticides in / on grape berries and leaves under Egyptian conditions. The objective of the present work was to study the dissipation and residue of chlorfenapyr and difenoconazole in/on grape berries and leaves also determination the pre-harvest intervals (PHI) corresponding to recommended rate of application.

**2. Experimental**

**2.1. Chemicals and reagents**

All reagent solvents were pesticides, HPLC or analytical grade, acetonitrile and methanol were purchased from Fischer Scientific (loughborough, UK). Acetone, dichloroethane, ethyl acetate, charcoal and sodium sulphate from El-Nasr pharmaceutical chemical company (Egypt). n-hexane, toluene and ammonium hydroxide from Sigma Aldrich (Stlouis, MO, USA). Magnesium oxide, Cellite® 545 and florisil (60-100 mesh) from Merck Ltd (UK). Chlorfenapyr and difenoconazole references standard were 99% purchased from Sigma Aldrich. Chlorfenapyr formulation (Challenger® 36% SC) from Basf Limited Egypt and difenoconazole formulation (Score® 25% SC) from Syngenta agro Egypt.

**2.2. Field Experiment and Sampling**

The cultivated area with grape (*vitisvinifera* L.) (1 Fadden) was at Shebin EL-kom, EL-Menoufia governorate, Egypt, contain eleven longitudinal lines 1.25 meters between each. The area was divided to three plots, and two randomized plots were treated with the tested pesticides formulations at the rate of 144 g.a.i/ha and 125 g.a.i/ha for chlorfenapyr and difenoconazole, respectively. One plot was left as a control without treatment. A knapsack sprayer equipped with one nozzle was used for applying formulations of tested pesticides after water dilution rate of 1000 L /ha.

All samples (2kg for each) were taken at random from each experimental plot according **FAO/WHO recommendations (1986).** Sub-sampling was done at the laboratory; three replicates were taken (50 g of berries and 20 g of leaves) for pesticide residue analysis. Sampling intervals were zero time (one hour), 1, 3, 5, 8, 12, 15, 18 and 22 days after application. The samples were preserved in a clean new polyethylene bags and stored at -20 °C in a deep freezer until residue analysis.

**2.3. Extraction and cleaning up**

Frozen samples of 50 g of grape berries and/or 20 g of leaves were extracted by acetone (150 mL) for 5 min. in a high speed blender, followed by partitioning using 100 mL of 10 % sodium chloride solution and 3x50 mL dichloromethane (**Abo El-soud, *et al*., 1995).** The collected organic layer was evaporated to dryness and re-dissolved in n-hexane for clean up. A glass column packed with 6 g activated florisil (60-100 mesh) and 1.5 g mixture of anhydrous sodium sulfate: activated charcoal (1.1: 0.4 w/w) on the top was used. Chlorfenapyr was eluted from the column with 100 mL of n-hexane: ethyl acetate (7:3 v/v) then the eluate was evaporated to dryness (**Papia *et al.,* 2010).**

Difenoconazole residues were extracted from the samples by 150 mL of (methanol: conc. Ammonium hydroxide) (80:20 v/v) for 5 min. at a high speed blender, followed by partitioning using 50 mL super saturated sodium chloride solution and 2x50 mL n-hexane. Organic layer was collected and re partitioned by 2x50 mL acetonitrile. Collected organic layer was evaporated and re dissolved in toluene for clean-up. A glass column packed with 7 g of activated charcoal: magnesium oxide:cellite (1:2:4 w/w/w) was used. Difenoconazole was eluted from the column with 120 mL of toluene: acetonitrile (1:1 v/v), then collected and evaporated to dryness (**Grunewald *et al*., 1993).**

Untreated samples of grape berries and leaves were spiked with known concentration of the tested pesticides (0.025, 0.05 and 0.1 mg/kg) to examine the eﬃcacy of extraction and clean up, table (1). Results were corrected according to the average of recovery.

**Table (1). Recovery rates of tested pesticides under study.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Fortification****Levels (mg/kg)\*** | **Recovery percent ± SD** |  |  |
| **Chlorfenapyr** |  | **Difenoconazole** |
|  | **Berries** | **Leaves** |  | **Berries** | **Leaves** |
| **0.025** | 91.69 ± 2.06 | 81.69 ± 3.59 |  | 93.31 ± 2.83 | 88.56 ± 4.95 |
| **0.05** | 83.07 ± 1.62 | 92.96 ± 2.40 |  | 94.34 ± 4.77 | 90.29 ± 3.71 |
| **0.1** | 89.37 ± 1.14 | 84.00 ± 4.34 |  | 91.22 ± 1.78 | 89.50 ± 2.15 |

\*Each fortification level is a mean of three replicates

**2.4. Residue determination**

Analysis of chlorfenapyr and difenoconazole was carried out using Agilent 7980 GC equipped with a micro electron capture detector (µ ECD). Compounds were separated on afused silica DB-1701 capillary column (30m x 0.32 mm) 0.25 µm film thickness, in combination with the following oven temperature 190°C, held for 2 min., 10 °C /min. ramp to 250°C held for 5 min. for chlorfenapyr determination, whereas for difenoconazole determination: initial temperature 245°C held for 2 min., 5°C/min. ramp to 260°C and held for 5 min., the carrier gas (nitrogen) flow rate was set to a constant of 4 mL/min. Injection port temperature and detector temperature were set at 220 °C and 300 °C, respectively. These conditions resulted in good separation and high sensitivity was obtained with retention time 8.2 min. and 6.3 min. for chlorfenapyr and difenoconazole, respectively.

* 1. **. Residue half-life estimation (t*0.5*)**

The half-life time (t*0.5*) for each investigated pesticides were calculated using the following equation of **moye*et al*., (1987).**

t*0.5 =* Ln 2/K = 0.6932/K

k´ = 1/Tx x Ln(a/bx)

|  |  |
| --- | --- |
| k´ = rate of decomposition | Tx = time in days |
| A = initial residue | Bx = residue at x time |

Whereas

**3. Results and Discussion**

* 1. **Dissipation rates of chlorfenapyr in/on grape berries and leaves.**

The initial residue deposits, dissipation rates,half-life (t0.5) of chlorfenapyr and difenoconazole in/on grape berries and leaves is shown in table (2 and 3).

Data in table (2) and figure (1) indicated the initial deposits of chlorfenapyr (one hour after application) in/on grape berries was 1.93± 0.31 mg/kg. The residues decreased consequently by times to 0.881± 0.14 mg/kgafter 24 hours with dissipation percent 54.17 %. The rate of dissipation was 67.91, 85.20, 90.69, 98.54, 99.74 and 99.79 % after 3, 5, 8, 12, 15 and 18 days. The residue of chlorfenapyr was undetected in grape berries after 22 days from application. Whereas the initial residue deposit of chlorfenapyr in leaves was 4.158 mg/kg. Consequently decreasing of the initial deposit with dissipation rate of 38.05, 60.80, 78.00, 84.75, 95.65, 99.57, 99.83 and 99.93% after 1, 3, 5, 8, 12, 15, 18 and 22 days from treatment.Plotting logarithm residue concentration (logmg/kg) against time (days after application), fig.(1), resulted best fitting regression coefficient (R2 = 0.98 and 0.97) for berries and leaves, respectively. The resulted data indicated the reaction kinetic of chlorfenapyr that obey fist order and agreed with **Papia*et al*.,(2010)** whose mentioned that chlorfenapyr was dissipated in chilli, cabbage and soil following first-order kinetics.

The calculated rate of dissipation and half-life (t0.5) were 0.386, 1.796 and 0.294, 2.36 for grape berries and leaves, respectively. The obtained data are harmonized with that obtained by (**Cao *et al.,* 2005; Yan-ling *et al.,* 2007and Papia *et al.,*2010).**

* 1. **Dissipation rates difenoconazole in/on grape berries and leaves.**

The initial deposits average of difenoconazole in/on grape berries, table (3) and figure (2) was 1.773 mg/kg. This amount dissipated after 24 hours to 1.362 mg/kgwith dissipation rate percent of 23.18 %. As time elapsed of difenoconazole residues dissipated by rates 42.02, 51.90, 66.89, 75.86, 92.44 and 97.85 % after 3, 5, 8, 12, 15 and 18 days from treatment. Whereas in leaves, the initial residue deposits in leaves was 3.642 mg/kg. This amount decreased to 2,988 mg/kgwith dissipation rate 17.96 % at 24 hours from treatment. Consequently dissipations by rates 33.44, 52.91, 63.18, 77.38, 85.75, 94.37 and 98.54 % at 3, 5, 8, 12, 18 and 22 days, respectively. The obtained data resulted best fitting regression coefficient (R2 = 0.915 and 0.943) for berries and leaves, respectively. As chlorfenapyr, difenoconazole dissipation rates obeyed first order kinetic reaction that agreed with **Wang *et al*., (2008).** The calculated dissipation rates of difenoconazole and half-life (t0.5) in grape berries and leaves were 0.154, 0.135 and 4.49, 5.13 days, respectively. Data obtained harmonized with **Ruilan *et al*. (2010),** whereas **Wang *et al*., (2008)** found that the half-life of difenoconazole in Chinese cabbage were 6.6 days in 2005 and 7.8 days in 2006.

The initial residue amounts of chlorfenapyr and difenoconazole in grape leaves were higher than grape berries, this attributed to the fact that; grape berries is covered by leaves, also morphological and physiological characteristic of berries and leaves greatly influence the distribution, retention and uptake of pesticides into their tissue (**Edward, 1975**). Chlorfenapyr was applied by rate (144 g.a.i/ha) whereas difenoconazole was applied by rate (125 g.a.i/ha), results show that high initial residues of chlorfenapyr comparing difenoconazoleresidues. Dissipation rates of tested pesticides were higher in grape berries than grape leaves. Chlorfenapyr dissipated by rates faster than difenoconazole in both berries and leaves, this is due to chlorfenapyr (limit systemic insecticide) is non-polar pesticides and their Octanol / Water partition coefficient (Kow= 4.83) and also have high vapor pressure (< 1.2 x 10-2mpa), whereas the Octanol / Water partition coefficient of difenoconazole (Kow = 4.4) which act as a systemic fungicides. The previous results gave explanation for the high persistence of difenoconazole residues in grape berries and leaves as comparing of chlorfenapyr residues.

The maximum residue limit (MRL) of chlorfenapyr in table grapes and leaves was 0.01 mg/kgas established by **European** **Commission Regulation (EU) 2013**. Accordingly, it is recommended that grape berries and leaves may be safely for consumption after 15 and 17 days, respectively. Whereas the MRL of difenoconazole as established by (**codex Alimentrius Commission 2013)** was 0.1 mg/kgin grape berries and leaves, accordingly the safely consumption of berries and leaves recommended to be after 17 and 21 days from spraying, respectively.

In conclusion this study evaluated the dissipation rates of chlorfenapyr and difenoconazole in grape berries and leaves. The results indicated that chlorfenapyr disappear rapidly in grape berries and leaves, and both pesticides exhibited first-order kinetics dissipation under the Egyptian field conditions. Also proposes the need of application of the safety periods (PHI) before harvesting and marketing grape berries and leaves.

**Table (2): Initial residue deposit and residue decline of Chlorfenapyr in/on grape berries and leaves.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Days after****application** | **Grape berries** |  | **Leaves** |
| **Means\*** | **% Dissipation** |  | **Means** | **% Dissipation** |
| **0** | 1.923 ± 0.31 | 00.00 |  | 4.158± 1.22 | 00.00 |
| **1** | 0.881 ± 0.14 | 54.17 |  | 2.576± 0.87 | 38.05 |
| **3** | 0.617 ± 0.08 | 67.91 |  | 1.630± 0.63 | 60.80 |
| **5** | 0.285 ± 0.05 | 85.20 |  | 0.915± 0.35 | 78.0 |
| **8** | 0.179 ± 0.11 | 90.69 |  | 0.634± 0.19 | 84.75 |
| **12** | 0.028 ± 0.02 | 98.54 |  | 0.181± 0.13 | 95.65 |
| **15** | 0.005 | 99.74 |  | 0.016± 0.02 | 99.57 |
| **18** | 0.004 | 99.79 |  | 0.007 | 99.83 |
| **22** | BLD | 100 |  | 0.003 | 99.39 |
| **MRL (mg/kg) \*\*** | **0.01** | **0.01** |
| **K´(days-1)** | **0.386** | **0.294** |
| ***t*0.5 (days)** | **1.796** | **2.359** |
| **R2** | **0.986** | **0.97** |
| **PHI (days)** | **15** | **17** |

\*Means = mg/kg ± S.D. Values given are the means of three replicates.

\*\* Maximum residue limit according to (European Commission Regulation (EU), 2013)

**Table (3): Initial residue deposit and residue decline of difenoconazole in/on grape berries and leaves.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Days after****application** | **Grape berries** |  | **Leaves** |
| **Means\*** | **% Dissipation** |  | **Means** | **% Dissipation** |
| **0** | 1.773 ± 0.49 | 00.00 |  | 3.642 ± 1.13 | 00.00 |
| **1** | 1.362 ± 0.18 | 23.18 |  | 2.988 ± 1.55 | 17.96 |
| **3** | 1.028 ± 0.23 | 42.02 |  | 2.424 ± 0.79 | 33.44 |
| **5** | 0.853 ± 0.11 | 51.90 |  | 1.715 ± 0.47 | 52.91 |
| **8** | 0.587 ± 0.25 | 66.89 |  | 1.341 ± 0.52 | 63.18 |
| **12** | 0.428 ± 0.08 | 75.86 |  | 0.823 ± 0.29 | 77.38 |
| **15** | 0.134 ± 0.03 | 92.44 |  | 0.519 ± 0.31 | 85.75 |
| **18** | 0.038 ±0.005 | 97.85 |  | 0.205 ± 0.14 | 94.37 |
| **22** | BLD | 100 |  | 0.053 ± 0.05 | 98.54 |
| **MRL (mg/kg) \*\*** | **0.1** | **0.1** |
| **K´(days-1)** | **0.154** | **0. 135** |
| ***t*0.5 (days)** | **4.494** | **5.134** |
| **R2** | **0.915** | **0.943** |
| **PHI (days)** | **17** | **21** |

\*Means = mg/kg ± S.D. Values given are the means of three replicates.

\*\* Maximum residue limit according to (**Codex Alimentarius Commission, 2013**)

**Fig. (1): Log. Residue- day regression line of chlorfenapyr residue in grape berries (A) and**

**Leaves(B).**

**Fig. (2): Log. Residue- day regression line of difenoconazole residue in grape berries (A) and**

**Leaves (B).**

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