

Dissipation behavior of Chlorfenapyr on Citrus and Tomato in Egypt

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ABSTRACT: Improper uses of pesticides leads to occupational, environmental and food security risks. Ethyl acetate followed by GC determination for the analysis of chlorfenapyr residues in orange and tomatoes were performed. Chlorfenapyr accuracy, repeatability, linearity and specificity were investigated. Pesticide regression coefficients (R(2)) were 0.9953 and 0.9901 in orange and tomatoes, respectively. LODs were 2.3 and 2.4 µg/kg, respectively, while LOQs were 8.0 and 7.8 µg/kg, respectively. Recovery% was ranged from 91.12% to 96.54%. The Half-life values (t_{1/2}) were 2.85 and 2.89 days for orange and tomato, respectively. The PHI was 21 and 10 days for orange and tomato, respectively. Detection of Chlorfenapyr PHI residues in this work highlights on the urgent needs for good implementation in Good Agriculture Practices and continuous control for these crops to minimize the health risk to consumption and improve exports.

KEYWORDS: Chlorfenapyr, Citrus, Tomato, Egypt.

1 INTRODUCTION

Despite its requirement for agricultural production, inappropriate use of pesticide will lead to environmental and food contamination. Hence, the use of pesticide should be under rigorous monitoring to ensure food safety (Kapoor *et al.* 2013).

Chlorfenapyr was found in a grapefruit sample imported from South Africa at 0.016 mg/kg (The MRL is 0.01 mg/kg). Chlorfenapyr was reported in imported nectarine sample from South Africa at 0.04 mg/kg (The MRL is 0.01 mg/kg). Also, Chlorfenapyr residue was isolated from granadilla sample from Colombia at 0.029 mg/kg (The MRL is 0.01 mg/kg), (Pesticides Residues in Food, 2014).

Oranges are one of the most exported fruits of Egypt, ranking second in the world in orange exports. Egyptian oranges are of the highest quality, thus accepted by all countries around the world such as the Russian Federation, Saudi Arabia, United Kingdom etc... Egypt orange exports have reached 1.1 million tons in 2013 (International Trade Center, 2015).

Chlorfenapyr is one of pesticides detected in exported European Union mandarins above the permissible limits (European Food Safety Authority, 2014).

The tomato is the edible, often red fruit/berry of the nightshade, commonly known as a tomato plant. They are harvested from January to December. Tomato, *Lycopersicon esculentum* is an important vegetable crop grown all over the world. Also, it is one of the first horticultural crops in Egypt, grown in covered greenhouses and in open fields. Many dangerous pests in tomato in recent years have been appearing (Barakat *et al.*, 2015). The pest has caused serious damages to tomato in invaded areas and it is currently considered a key agricultural threat to European and North African tomato production (Desneux *et al.*, 2010 and Garcia and Vercher, 2010).

On the other side, chemical pesticides remain to be having an important role in insect pest management even with the improvement of other control methods as mass-trapping, plant resistance...etc. Generally, pesticides play an important role

in increasing agricultural production, but their random use causes destructive effects on human health (Soomro *et al.*, 2008) in addition to the untargeted organisms (Akobundu, 1987) and finally distribute the agri-system and environment. Ninety nine percent of all poisoning cases occur in developing countries. The farmers in the fields are at high risk of being poisoned (UNDP, 2001).

Uses of pesticides depend on different chemistries and with different modes of action is an important module for integrated pest management strategy. Hence, pesticides will continue to be an integral constituent of pest management programs due to their effectiveness and simple use (Braham and Hajji, 2011).

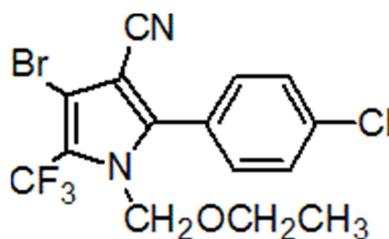
So, this study aimed to determine the dissipation rate, half-life values (t_{50}) and pre-harvest interval (PHI) for the two studied crops.

2 MATERIAL AND METHODS

2.1 PESTICIDE

Chlorfenapyr is a pesticide, and a pro-insecticide (meaning it is metabolized into an active insecticide after entering the host), derived from a class of microbially produced compounds known as halogenated pyrroles (US EPA, 2001).

Chemical Name: 4-bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-pyrrole-3-carbonitrile



Structural Formula

2.2 CHEMICALS AND REAGENTS

All reagent solvents were HPLC grade. Ethyl acetate, Sodium bicarbonate and sodium sulphate from El-Nasr pharmaceutical chemical company (Egypt). Chlorfenapyr references standard were 99% purchased from Sigma Aldrich. Chlorfenapyr formulation (Challenger super® SC 24% WV) from Basf Limited Egypt.

2.3 FIELD EXPERIMENT

The cultivated area with citrus (orange) and tomato (1 Fadden each) was at Qaluobia district, Egypt. The tested area was subdivided to three parts, and two randomized sites were treated with the tested pesticides formulations at the rate of 144 g.a.i/ha for chlorfenapyr. 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.

2.4 SAMPLING

Sampling was randomly collecting from various places of the experimental plots according to the **FAO/WHO recommendations (1986)**. 3 replicate samples about 2.5 kg of orange and tomato were collected from pesticide-treated fields. Samples were taken 2 h after the pesticide application. Subsequent samples were taken at 1, 3, 7, 10, 15, and 21 days after treatment. During experiment, a control sample was taken in each sampling time. After samples collection, samples were packed in plastic bags and transported to the laboratory in an ice box. The samples were homogenized using a food processor (Thermomix, Vorwerk). Each sample homogenate was done where three representative samples of 15 g were taken. Then, samples were placed into polyethylene 50 ml centrifuge tube and frozen at -20°C until analysis.

2.5 EXTRACTION (EURL-FV, 2010)

- 1) Weigh 3.0 g sodium hydrogen carbonate (NaHCO₃) to 10 ± 0.1g sample in a 50 m. centrifuge tube.
- 2) Add 10 g sodium sulphate (Na₂SO₄) and 20 ± 0.1ml ethyl acetate and extract by shaking (30 sec) and ultrasonication (3 min), max 35°C.
- 3) Centrifuge 3 minutes at 3200 g.
- 4) Filtrate the crude extract through a 0.20 µm PTFE filter.
- 5) Inject to GC for analysis of dried fruits (water content of Ca 20%) addition of water is needed before extraction. Add 750 g cold water (4°C) to 500 g dried fruits and homogenate the sample. Weigh 25 g sample corresponding 10 g dried fruit and add 20 ml ethyl acetate.

2.6 INSTRUMENT AND INJECTION CONDITION

Gas chromatography (Hewlett Packard 7890) with a microelectron capture detector (µECD) was used.

Column: HB-5 capillary column (30 m x 0.25 mm, µm film thickness).

Nitrogen was the gas carrier.

Inlet 250°C

Flow rate: 3 ml/ min

Detector temperature: 220 °C

RT: 6 min

Condition: The temperature program was 200°C for 1 minute, then increase 5°C/minute till become 280°C and hold for 5 minutes.

2.7 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)**.

$$\text{Half-life} = \frac{\text{Elapsed time} \times \log 2}{\log \left[\frac{\text{Beginning amount}}{\text{Ending amount}} \right]}$$

2.8 STATISTICAL ANALYSIS

Data obtained were statistically analysed for descriptive statistics, Q squares and ANOVA test at significant level of P < 0.05 using **SPSS 14 (2006)**.

3 RESULTS AND DISCUSSION

METHOD VALIDATION

The residue analysis in orange and tomato matrices and detection of Chlorfenapyr using gas chromatography were performed. Recoveries were measured by comparing peak areas of the spiked samples with external standards in ethyl acetate and matrices matched control prepared from unfortified extracts. The specificity of method indicated that there was no interference from solvent and matrix. Quantification was performed using standard curve prepared by stock solution in ethyl acetate. Good linearity was done between the ranges of 0.01 to 1.0 µg/ml with a correlation coefficient of 0.9994 (Fig., 1).

Precision of the method was determined by analyzing the orange and tomato samples at the 1.0 mg/kg spiked level with six replicates by different analyst and different day. Mean recoveries of Chlorfenapyr at difference fortification levels validated the procedures adopted for extraction and analysis of Chlorfenapyr residues from orange and tomato samples. The mean recovery achieved were 91.12% and 96.54% for orange and tomato, respectively. LODs were recorded to be 2.3 and 2.4 µg/kg for orange and tomatoes, respectively. LOQs were 8.0 and 7.8 µg/kg for orange and tomatoes, respectively (Table, 1). These results was nearly similar to the earlier studies indicated that the mean recovery of Chlorfenapyr in orange and tomatoes were 89% and 93% as well as LOQs were between 0.05 and 0.01 mg/kg using GC-ECD detector (Kim, 1994, Lindsell, 1994, EFSA and 2013). Also, these results are similar to that reported by Moi *et al.*, (2007) but on GC-MS and LC MSMS.

Table (1): Parameter of Chlorfenapyr analytical method validation.

| Parameter | Orange | Tomato |
|------------|-----------|-----------|
| LODs | 2.3 µg/kg | 2.4 µg/kg |
| LOQs | 8.0 µg/kg | 7.8 µg/kg |
| Recovery % | 91.12% | 96.54% |

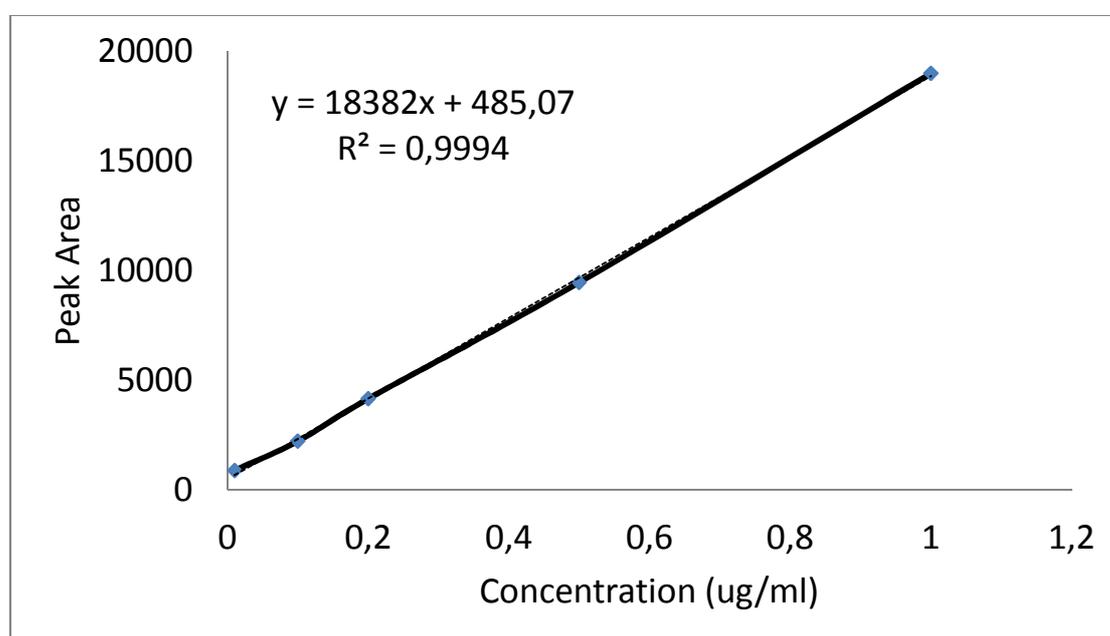


Fig. (1): Calibration curve for Chlorfenapyr in ethyl acetate.

DISSIPATION OF CHLORFENAPYR IN ORANGE

The dissipation rate of chlorfenapyr in orange was exhibited first order kinetics. The regression equations and half-life value for chlorfenapyr are summarized in Table (2) and Fig. (2).

The Half-life values ($t_{1/2}$) were calculated mathematically and graphically to be 2.857 days (2 days and 20.58 hour). This observation was similar to that reported by Fantke *et al.* (2014) who recorded that the half-life of chlorfenapyr in plant was ranged from 2.35–4.86 day. Otherwise, it is lower than that reported by Lim *et al.* (2011) who recorded that the biological half-lives of chlorfenapyr was about 3.5 days in Chwinamul. While, the half-life in this study was higher than those recorded for Grape (1.796 day) by Abdallah *et al.* (2014). The differences in recorded half-life could be regarded to difference in cultivation plant and/or temperature, or climate changes during spraying.

In this study, the PHI of chlorfenapyr in orange was calculated to be 21 Days (Table, 2). This observation become orange safe for human consumption and exportation after 21 days of application where the residue level was equal to the maximum residue level (0.01 mg/ kg) recorded by EU (2005a).

The residue level of chlorfenapyr in orange samples obtained from this experiment conducted at Qaluobia district, Egypt, in summer season, at August 2015, the mean sunshine period during the experiment was about 13 h /day while the maximum temperature ranged between 38°C and 41°C during 0 to 21 days after application.

The prolonged sunshine hours and higher temperature in summer period (with low moisture) at Qaluobia district could be favoured faster degradation of chlorfenapyr pesticide. These results parallel to that obtained by **Kandil et al. (2011)**, who concluded that the residues of chlorfenapyr were greatly deteriorated when exposed to UV-Rays sunlight and high temperature. The most prominent effect was related to the impact of UV-rays incomparable to direct sunlight.

Table 2: Dissipation of Chlorfenapyr residue in Orange

| Time after treatment (days) | Residues (mg/kg), | Dissipation % | Persistence |
|-----------------------------|-------------------------|---------------|-------------|
| Initial * | 1.63 ± 0.13** | - | 100 |
| 1 | 1.2 ± 0.14 | 26.38 | 73.61 |
| 3 | 0.71 ± 0.08 | 40.83 | 65.4 |
| 7 | 0.3 ± 0.04 | 57.74 | 18.4 |
| 10 | 0.15 ± 0.011 | 95.1 | 9.2 |
| 15 | 0.06 ± 0.01 | 96.3 | 3,7 |
| 21 | 0.01 ± 0.008 | 99.4 | 0.6 |
| PHI | 21 days | | |
| t _{0.5} (days) | 2.857 days | | |
| MRL | 0.01 mg/ kg (EU, 2005a) | | |

Initial *: one hour after application

** Data represented as mean ± SE

LOD 0.05 mg/kg

HALF-LIFE CALCULATION IN ORANGE

1. MATHEMATICALLY

According to equation

$$\text{Half-life} = \frac{\text{Elapsed time} \times \log 2}{\log \left[\frac{\text{Beginning amount}}{\text{Ending amount}} \right]}$$

$$\text{Half-life} = \frac{21 \times 0.301}{2.212} = 2.857 \text{ days} = 2 \text{ days and } 20.58 \text{ hour}$$

2. GRAPHICALLY

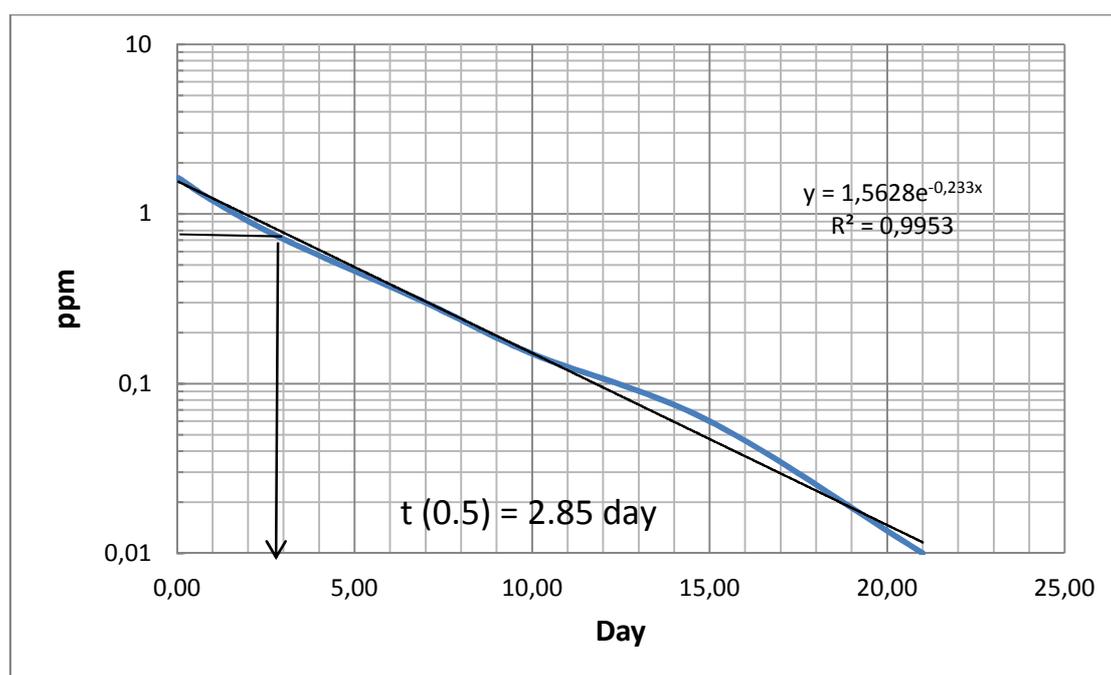


Fig. (2) Decline in chlorfenapyr residues over time following its application to orange and t-half graphically on semi log pattern

DISSIPATION OF CHLORFENAPYR IN TOMATOES

Pattern of dissipation of chlorfenapyr in tomato followed first order kinetics also. The half-life was calculated mathematically and graphically using semi-log technique to be 2 days and 21.37 hour (Table, 3 and Fig., 3). This result is nearly similar to that obtained in Orange in this study. This could be explained by the fact that the two studies were performed in the same area and time (climate condition).

In this study, the calculated PHI was 10 days where its residue level reaches to 0.01 mg/kg, according to European Union MRL (EU, 2005b). This result differ than that of *Galiotta et al. (2011)* who recorded that 16 days after application are necessary to meet the European MRL requirements for chlorfenapyr in tomato.

The timeout of chlorfenapyr in tomato in Egypt is of 10 days. Although Codex Alimentarius does not have an established MRL value, while the EU established 0.01 mg/Kg. According to the mathematical model adjusted, 10 days after application are necessary to meet the European MRL requirements.

This result confirms that the residues of chlorfenapyr were greatly deteriorated when exposed to direct sunlight with its ultraviolet light (UV), especially with the prolongation of the sunlight exposure period (*Kandil et al., 2011*). Taking in consideration that, the study was performed during September with sunlight period about 13 hours and temperature ranged between 37 and 41°C. In addition to, tomato has a smooth surface, facilitate the pesticide dropping from tomato fruit. Results further refer that the rate of degradation of chlorfenapyr varied according to period of exposure to direct sunlight.

Generally speaking the effect of ultraviolet light (UV) on the degradation of pesticides is of considerable interest to scientists in that field, UV-rays exerts chemicals changes on chlorfenapyr pesticide such as hydrolysis, oxidation and isomerization. When such reactions occur under field conditions, they induce considerable environmental contamination and produce serious pesticide residues on and in agricultural products (*Hou et al., 2005* and *Kandil et al., 2011*).

This appears to be the reason for the lower level residues on '0' day at Qaluobia district, where the chemical was exposed to sunlight for a longer period. This study proved that residue of chlorfenapyr when sprayed at the recommended dosage will become safe after 10th day where tomato samples were lower than 0.01 mg/Kg.

Table 3 : Dissipation of Chlorfenapyr residue in Tomato

| Time after treatment (days) | Residues (mg/kg) | Dissipation % | Persistence |
|-----------------------------|-------------------------|---------------|-------------|
| Initial * | 0.11 ± 0.05 | - | 100 |
| 1 | 0.084 ± 0.02 | 23.63 | 76.36 |
| 3 | 0.055 ± 0.01 | 34.6 | 65.4 |
| 7 | 0.024 ± 0.01 | 81.6 | 18.4 |
| 10 | 0.01 ± 0.002 | 90.9 | 9.09 |
| 15 | 0.005 ± 0.001 | 96.3 | 3.7 |
| 21 | ND | - | 0.6 |
| PHI | 10 days | | |
| t _{0.5} (days) | 2.89 Day | | |
| MRL | 0.01 mg/ kg (EU, 2005b) | | |

Initial *: one hour after application

** Data represented as mean ± SE

ND: Non Detectable

HALF-LIFE CALCULATION IN TOMATO:

1- MATHEMATICALLY

$$\text{Half-life} = \frac{10 \times 0.301}{1.041} = 2.89 \text{ days} = 2 \text{ days and } 21.37 \text{ hour}$$

2-GRAPHICALLY

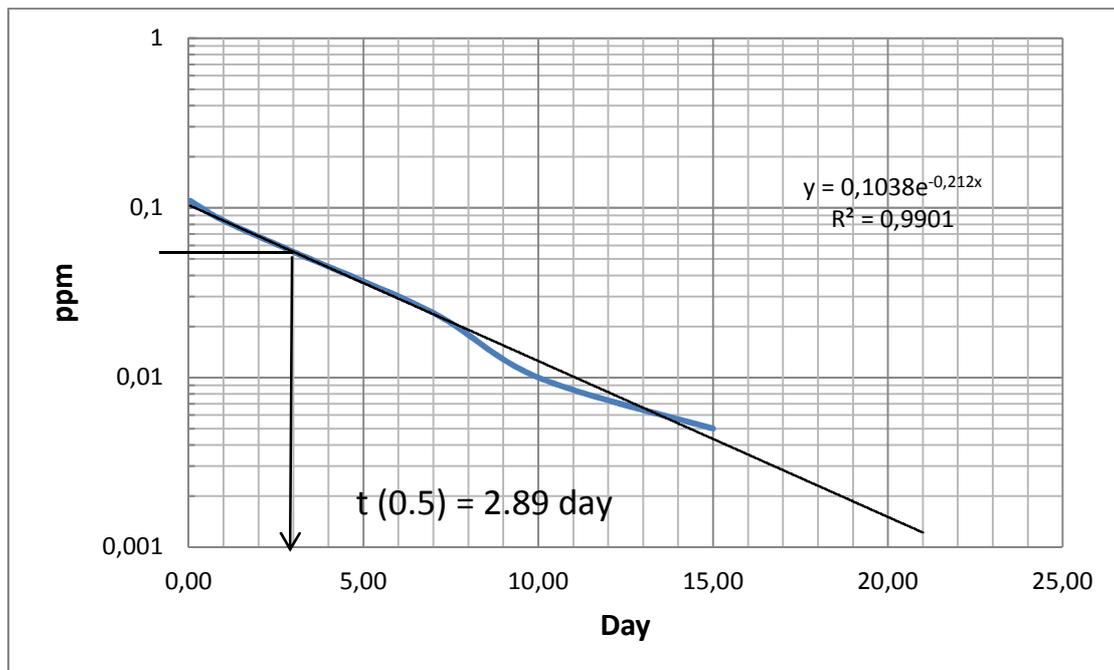


Fig. (3) Decline in chlorfenapyr residues over time following its application to tomato and t-half graphically on semi log pattern

4 CONCLUSION

The time out of chlorfenapyr for orange to be exported to the EU must be reviewed in Egypt in order to meet the MRL requirements. The EU has a MRL of 0.01 mg/kg (EU, 2005a), therefore to meet such condition, according to our experimental data, 21 days after application should be required.

Residue levels of chlorfenapyr in tomato proved that, the required time for crop clearance was 10 days according to EU MRL requirements (0.01 mg/kg) (EU, 2012).

Our results reinforce the significant in order to improve dissipation curves for the registered pesticides in Egypt under local temperature and conditions to escape problems that may cause the rejection of the exported economical crops.

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