Middle East Journal of Agriculture Research
ISSN 2077-4605

Volume : 06 | Issue : 01 | Jan.-Mar. | 2017
Pages:135-142

Residues Assessment of Captan, Spirodiclofen and Thiophanate Methyl in Apple Fruits under the Field Conditions

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Received: 15 December 2016 / Accepted: 20 January 2017 / Publication Date: 21 March 2017

ABSTRACT

Dissipation behavior and residual levels of three pesticides (captan, spirodiclofen, and thiophanate methyl) applied to apple trees were studied using QuEChERS extraction method under climatic conditions in Egypt. The residues of tested pesticides were determined after one hour, 1, 3, 7, 10 and 15 days. The apple samples of captan, spirodiclofen, and thiophanate methyl contained residues lower than the maximum residue limits (MRL) (3, 0.8 and 0.5 mg/kg) so that the apples fruits could be used safely at any time after 3 days of application. The half-life values ($t_{1/2}$) were 2.47, 0.91 and 1.09 days for captan, spirodiclofen and thiophanate methyl, respectively.

Key words: Dissipation, residues, captan, spirodiclofen and thiophanate methyl, MRL and half-life.

Introduction

The presence of pesticide residues in food is one important concern for consumers, due to their possible adverse health effects. Various international organizations (Environmental Protection Agency (EPA), Codex Alimentarius Commission, World Health Organization (WHO) and Food and Agriculture Organization (FAO) of the United Nations) have regulated the use of pesticides, by fixing maximum residue levels (MRL's) for commercial purposes. The maximum residue levels (MRLs) are the highest levels of residues expected to be in the food when the pesticide is used according to authorized agricultural practices. Government agencies and international organization limit the amount of pesticides in food establishing maximum residue limits, with the aim of protecting consumers health (EFSA, 2010), so in recent years, the established regulations regarding the maximum residue levels (MRLs) have become more and more harmful. The European Union (EU) has set new directives for pesticides at low levels in vegetables in order to meet these health concerns.

Pesticides are extensively used to control pests and prevent diseases in various crops, such as fruits, vegetables and cereals. Chromatographic methods including gas chromatography (GC) and high-performance liquid chromatography (HPLC) have been widely used for the separation and quantification of pesticide residues in various samples such as vegetables and fruit samples, (Zhao *et al*. 2011 and Sharma *et al.* 2010)

Captan is a fungicide used for the control of fungal diseases in crops. Frequently used in orchards, and was selected as a marker of fungicide exposure. Captan had become the second most abundantly applied fungicide on apples and peaches (Hines *et al*., 2008). Captan is utilized by both home and agricultural growers and is often applied during apple production.Spirodiclofen is a tetronic acid with acaricidal action. It acts by interfering with mite development, thereby controlling such pests as *Panonychus spp*., *Phyllocoptruta spp*., *Brevipalpus spp*., and Aculus and Tetranychus species. Spirodiclofen is active by contact to mite eggs, all nymphal stages, and adult females (adult males are not affected). It controls mites in citrus, grapefruit, lemon, lime, calamondin, citrus citron, citrus hybrids, kumquat, mandarin, grapes, pome fruit (apple, crabapple, loquat, mayhaw, pear, oriental pear, quince), stone fruit (apricot, cherry, nectarine, peach, plum, plumcot, prune), and tree nut crops (almond, beechnut, Brazil nut, butternut, cashew, chestnut, chinquapin, filbert, hickory nut, macadamia nut, pecan, pistachio, walnut).Thiophanate-methyl is a member of the benzimidazole group of fungicides. It is a broad-spectrum systemic fungicide with protective and curative action, and absorbed by the roots and leaves. It is effective against a wide range of fungal diseases in a number of crops.

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Apple (*M. domestica*) is a product of economic importance in the world. According to FAO statistics, apple production in the world was realized in 4.7 million hectar area. In the same year, the apple production in the world was 75.4 million tones. Apple orchard has been planted in large areas in Egypt. Pest and disease pressure varies considerably from year to year and this, consequently, affects requirements for apple trees protection. Besides a variety of insects such as codling moth (*Cydia pomonella*), sawfly insects (*Hoplocampa testudinea*), tortricid (Tortricidae), aphids (*Dysaphis plantaginea*) and fruit tree red spider mite (*Panonychus ulmi*) controlled within the pre-harvest time by organophosphates, carbamates and other insecticides, development of fungal diseases, namely apple scab (*Venturia inaequalis*), powdery mildew (*Podosphaera leucotricha*) and apple canker (*Nectria galligena*) has to be prevented during vegetation period by suitable fungicide preparations (Ticha *et al.,* 2008).

The present work was carried out to study the persistence of three pesticides; captan, spirodiclofen and thiophanate methyl in apple fruits under the normal field conditions, in order to protect the consumer, by recommending a waiting period from treatment to harvest.

Materials and Methods

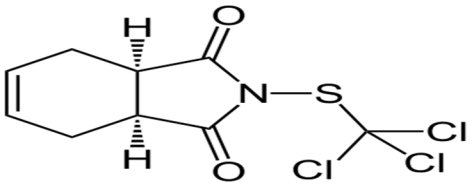
Chemicals and reagents:

Tested pesticides standards were obtained from the (CAPL) Central Agricultural Pesticide Laboratory (Dokki, Egypt), and purity was certified by the supplier to be ranged between 98.2 to 99.5%. All organic solvents used in the study were HPLC grade and supplied by Alliance Bio, USA. Primary and secondary amine (PSA, 40 µm Bondesil) was purchased from Supelco, Bellefonte, USA. Anhydrous Magnesium sulfate was of analytical grade, purchased from Merck and was activated by heating at 250 °C for 4 h, cooled and kept in desiccators before use. Trisodium citrate dehydrate and disodium hydrogen citrate sesquihydrat were purchased from Sigma Aldrich. Sodium chloride was of analytical grade and purchased from El Naser Pharmaceutical Chemicals Company and was activated by heating in the oven before use over night at 135°C.

Pesticides:

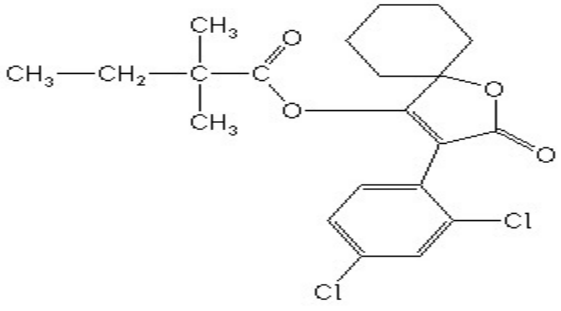
1- Captan:

N-trichlormethylmercapto-4-cyclohexen-1,2-dicarboximid.



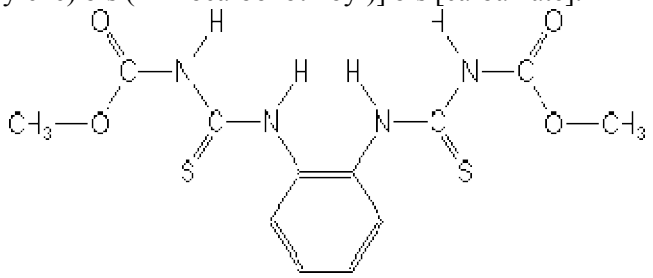
2- Spirodiclofen:

3-(2,4-dichlorophenyl)-2-oxo-1-oxaspiro[4.5]dec-3-en-4-yl2,2-dimethylbutanoate.



3- Thiophanate methyl:

Dimethyl [(1, 2-phenylene) bis (iminocarbonothioyl)] bis [carbamate].



Field experiments:

The field trails including the dissipation study and final residue study were conducted in a randomized block design in farmers' fields, which were previously investigated to be free of the tested pesticides. The supervised field trials were carried out in El-Qalubia Governorate, Egypt, during june 2015. Field trials were conducted in separate plots measuring 150 m^2 each in three replicates. None of the plots had been treated with tested pesticides. The commercial formulations of spirodiclofen (Envidor 24% SC), captan (Captan ultra 50% WP) and thiophanate methyl (Thiodine 70% WP) were applied on apple at 25 cm^3/100 L, 200g/100 L. and 65 g/100L., respectively. Apple samples (2 kg) were collected on 0, 1, 3, 7, 10 and 15 day after treatment. One untreated plot was left to serve as control. There was no rainfall at any time during the experimental period. The average daily temperature during the experiment ranged from 21 to 36 ºC the average relative humidity was 49 %. During experiment, a control sample was taken at each sampling time.

Sampling storage:

Immediately after collection the samples, each individual sample was put into plastic bags and transported in darkness in labeled polyethylene bags to the laboratory in an ice box and stored at -20 ºC until analysis.

Analytical methods:

The apple samples were homogenized in a food processor and 10 g of the homogenate of each sample were placed into 50-mL centrifuge tube.

Sample extraction and cleanup of captan, spirodiclofen and thiophanate methyl:

Apple sample (10 g) was transferred to a polyethylene (PFTE) 50-ml tube; 10 ml acetonitrile was added and shaken vigorously for 1 min. Then, buffer-salt-mixture (4 g of magnesium sulfate anhydrous, 1 g of sodium chloride, 1 g trisodium citrate dehydrate and 0.5 g disodium hydrogen citrate sesquihydrat) was added and shaken immediately for 1 min. Centrifugation was carried out at 4,000 rpm for 5 min. Supernatant (4 ml) of the clear solution was transferred to a new clean 15-mL centrifuge tube and cleaned up by dispersive solid-phase extraction with 100 mg PSA and 600 mg $MgSO_4$. The sample was again vortexed for 1 min and then centrifugation was carried out as mentioned above. The captan was analysed by GC-ECD, 2 mL of the supernatant were taken into a test tube, evaporated to dryness. 1 ml of hexane were added and transferred to glass vial for GCD-ECD. While the spirodiclofen and thiophanate methyl were analysed using HPLC-DAD, filtered 1 ml of supernatant into an injection vial to injecte in HPLC, (DIN EN 15662, 2009)

Method validation:

Validation of the analytical method was performed on parameters of linearity, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy. Linearity was determined by constructing calibration curves using standard solutions of different concentrations. The LOD was

determined as the lowest concentration giving a response three times the standard deviation of the baseline noise. The LOQ was determined as the lowest concentration of a given compound giving a response that was greater than 10 times the signal-to-noise ratio, with relative standard deviation lower than 20%. The accuracy of the method was checked by routine recovery assay at three levels of fortification (0.01, 0.05 and 0.5 µg/g), of captan, spirodiclofen and thiophanate methyl in apple fruits replicated five times alongside a control. The fortified samples were equilibrated and processed by adopting the method standardized in the present study. The precision of the method was determined through the relative standard deviation (RSD%).

Table 1: Recoveries of captan, spirodiclofen and thiophanate methyl in apple at various fortification levels.

Fortified level (mg/ kg) (na =5)	Recovery (%) of ± RSD		
	Captan	Spirodiclofen	Thiophanate methyl
0.01	80.5 ±2.3	78.56 ±2.85	79.14±3.7
0.05	86.2 ±2.8	84.25 ±3.1	82.6 ±3.9
0.5	95.1 ±3.1	93.7 ±3.5	90.4 ±2.9

[a]*Number of replicate.*

Data analysis:

The dissipation process follows first-order kinetics. The dissipation rate constant and half-life were calculated using the first order rate equation: $Ct = C_0e^{-kt}$, where Ct represents the concentration of the pesticide residue at the time t, C_0 represents the initial concentration after application and k is the dissipation degradation rate constant ($days^{-1}$). The half-life ($t_{1/2}$) was calculated from the k value for each experiment ($t_{1/2}= ln2/k$) (Moye *et al*. 1987).

Instrumental determination:

Captan determination:

The Agilent 6890 gas chromatograph equipped with an HP7673 auto-sampler, an electron-capture detector. A 30 m x 0.32 mm capillary column coated with a 0.25 µm thick film of 5% phenylmethylpolysiloxane (PAS-5). GC operating condition Injector and detector temperature were 280 and 300ºC, respectively and column temperature was 200 ºC. Flow rate was3 ml/min and retention time is 4.72 min.

Thiophanate methyl and spirodiclofen determination:

High Performance Liquid Chromatography Agilent 1100 series. The U.V. Diod array detector. The analytical column Nucleosil-C18, 5um (4 x 250 mm) was used.

The mobile phase for thiophanate methyl was acetonitrile: water (65/35 v/v) at flow rate 1 ml/min with wavelength 215 nm and the retention time is 2.32 min.

The mobil phase for spirodiclofen (acetonitrile 30 % + water 70 %). wavelength 246 nm. Flow rate: 1 ml/min and retention time is 6.02 min.

Results and Discussion

Method validation:

For method validation, calibration graphs were obtained by plotting the peak area of the quantification compound versus concentration in the range 0.01, 0.05, 0.1, 0.5 and 1 µg/ml. The correlation coefficient (r^2) was over 0.997 for three pesticides. Moreover, the fortified samples were analyzed under the same conditions. The precision was assessed by performing five repetitive determinations at each level. The results are shown in Table (1). The average recoveries of captan at 0.01, 0.05 and 0.5were 80.5%, 86.2% and 95.1%, respectively. While the average recoveries of spirodiclofen at 0.01, 0.05 and 0.5 were 78.56%, 84.25% and 93.7%, respectively. The average

recoveries of thiophanate methyl at 0.01, 0.05 and 0.5 were 79.14%, 82.6% and 90.4%, respectively. According to document No. SANCO/12495/2011 (European Commission 2011) the recovery range should be 70% - 120%, and relative standard deviation (RSD) should be <20%. The results in this study are within the accepted range for residue determination.

The limit of detection (LOD) and LOQ were defined, respectively, as the signal corresponding to 3 and 10 times the noise ratio, determined experimentally from fortified samples. In this study, the LOD and LOQ were estimated to be 0.005 and 0.01 mg/kg, for captan. While for spirodiclofen and thiophanate methyl were 0.01 and 0.01. The LOQ established allow for the identification and quantification of target analyte below the maximal residue limit established by the (European Union 2015) (3, 0.8 and 0.5 mg/kg) in apple fruits.

Dissipation and residue analysis:

The dissipation results of captan in apple are summarized in Table 2 and Fig. 1 the initial deposit (one hour after treatment) of captan in apple fruits was 7.32 mg/kg this value begins to decrease after one day to 5.12 mg/kg, it recorded 30.05 % loss. On the third day after application the residues were 2.89 mg/kg with the loss 60.51% in apple fruits after application. Seven days after applying the value of the residues was reduced to 0.51 mg/kg and the loss of the captan was 93.03 %. The residues of captan were reduced to 0.23 mg/kg and 0.15 mg/kg at the 10 and 15 days after spray, respectively. Determined residues of captan in apple fruits were at level below the maximum residue limits (MRL) 3 mg/kg (EU, 2016), so that the apple fruits could be used safely after 3 days from the spray by captan. These results were in agreement with Česnik *et al*., 2012. The half-life value of captan in apple fruits was 2.47 days.

Table 2: Residues of captan insecticide in apple fruits

Time intervals (days)	Residues (mg/kg) ±SE	Loss (%)	Persistence (%)
Initial*	7.32±0.12	0.00	100
1	5.12±0.25	30.05	69.95
3	2.89±0.07	60.51	39.49
7	0.51±0.09	93.03	6.97
10	0.23±0.016	96.85	3.15
15	0.15±0.014	97.95	2.05
$t_{1/2}$ (days)	2.47		
MRL(mg/kg)	3		

** Samples were taken after one hour of spraying.*
MRL = Maximum Residue Limit (EU 2016)
$t_{1/2}$ (days) = residue half- life.

Several factors may affect the persistence of pesticides as well as the effect of some physical and chemical factors like light, heat, pH, and moisture; some researchers reported that growth dilution factor might have played a significant role (Wang, *et al.,* 2007; Malhat & Hassan, 2011; Malhat *et al.,* 2013).

Also the residues of spirodiclofen in apple fruits summarized in Table 3 and Fig 1. The data showed that the residue in the initial deposit was 2.22 mg/kg, one hour after treatment in apple fruits. The amount of residues decreased to 1.01 mg/kg, this value gave the rate of loss 54.50 % within the first 24 hours after spray. The values of the residues decreased to 0.12 mg/kg after 3 days after application, it gave the rate of loss 94.59 %. Also, after 7 days the residues reduced to 0.07 mg/kg and gave 96.84 % loss of the spirodiclofen. At the 10 days after treatment the decreased of the values were reached to 0.02 mg/kg and the corresponding calculated rates of loss were 99.09 %. While, the samples taken 15 days after treatment contained no detectable amount of spirodiclofen. Determined residues of spirodiclofen insecticide in apple fruits were at level below the maximum residue limits (MRL), 0.8 mg/kg (EU, 2016), so that the apples fruits could be used safely after 3 after spray. These results were in agreement with Česnik *et al*., 2012. The half-life of spirodiclofen insecticide in apple fruits was 0.91 days.

Table 3: Residues of spirodiclofen in apple fruits.

Time intervals (days)	Residues (mg/kg) ±SE	Loss (%)	Persistence (%)
Initial*	2.22±0.14	0.00	100
1	1.01±0.06	54.50	54.50
3	0.12±0.13	94.59	5.41
7	0.07±0.05	96.84	3.16
10	0.02±0.07	99.09	0.91
15	ND	-	-
$t_{1/2}$ (days)	0.91		
MRL(mg/kg)	0.8		

** Samples were taken after one hour of application*
ND = Non detectable
MRL = Maximum Residue Limit (EU 2016).
$t_{1/2}$ (days) = residue half-life.

Thiophanate methyl residues in apple fruits showed in Table 4 and Fig 1, the residues in the initial deposit was 3.34 mg/kg with no loss in the thiophanate methyl. After 1 day from treatment the value begins to decrease to 1.82 mg/kg, it recorded 45.50 % loss. In the 3 days after application the residues of thiophanate methyl reduce to 0.49 mg/kg, it gave 85.32 % loss in the thiophanate methyl. Also, the decreased in the value of thiophanate methyl residues in apple fruits were continue in 7 and 10 days after spray that the values reached to 0.18 and 0.10 mg/kg, respectively, with 94.61 & 97.0 % loss, respectively, these results were in agreement with Abdel Megeed *et al*. 2000 and Dutta *et al.* 1995. While, the samples of apples fruits were taken 15 days after spray contained no detectable amounts of thiophanate methyl. Mandal *et al*. (2010) showed that thiophanate methyl residue in grapes was not detected in all the samples collected at 15th day after application irrespective of any location. Determined residues of thiophanate methyl residues in apple fruits were at level below the maximum residue limits (MRL) 0.5 mg/kg(EU, 2016), so that the apple fruits could be used safely after 3 days from the spray by thiophanate methyl, These results were in agreement with Jabr *et al*. (2014). The half-life of thiophanate methyl in apple fruits was 1.09 days.

The data obtaind revealed a higher initial deposits of captan compared with thiophenate methyl and spirodiclofen, indicating the correlatin between the concentration applied and the amount of residue deposited.

Table 4: Residues of thiophanate methyl insecticide in apple fruits.

Time intervals (days)	Residues(mg/kg) ±SE	Loss (%)	Persistenc (%)
Initial*	3.34±0.01	0.00	100
1	1.82±0.08	45.50	54.50
3	0.49±0.21	85.32	14.68
7	0.18±0.14	94.61	5.39
10	0.10±0.03	97.00	3.00
15	ND	-	-
$t_{1/2}$ (days)	1.09		
MRL mg/kg	0.5		

** Samples were taken after one hour of spraying.*
ND = Non detectable
MRL = Maximum Residue Limit (EU 2016).
$t_{1/2}$ (days) = residue half- life.

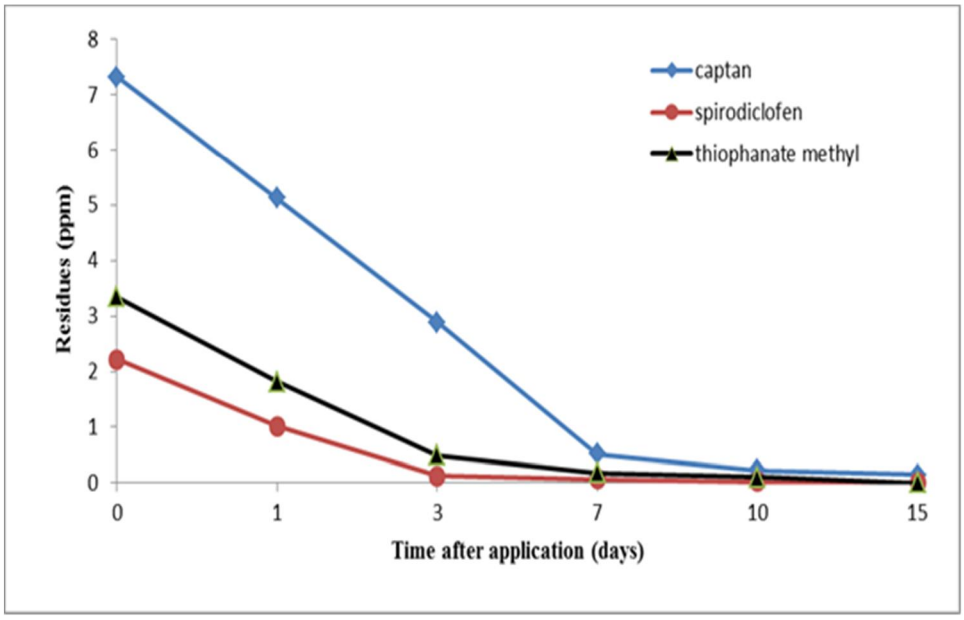


Fig. 1: Residues of captan, spirodiclofen and thiophanate methyl in apple fruits.

Conclusion

Residue levels of captan, spirodiclofen and thiophenate methyl in apple proved that, the required time for crop clearance was 3 days according to EU MRL requirements were 3, 0.8 and 0.5 mg/kg (EU, 2016).

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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