

Determination of Spinetoram Residues degradation in Tomato Fruits Using High Performance Liquid Chromatography (HPLC) and QuEChERS Method

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ABSTRACT: QuEChERS method and HPLC were used to trace the recovery and the residue of Spinetoram (insecticide mixture of XDE-175-J and XDE-175-L, EPA Chemical Code: 110008 and 110009) which applied against the tomato leaf miner, *Tuta absoluta* (Meyrick) on or in tomato fruits. The experiment was carried out at El-Kharga Oasis New Valley, Egypt during early summer plantation of 2013. 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 -7.5% in tomato with fortification levels of 0.1, 0.5 and 1.0 mg / kg⁻¹ respectively. Limit of quantification (LOQ) of this method was found to be 0.1 mg kg⁻¹, while limit of detection was 0.005 mg kg⁻¹. Half-life (t_{1/2}) and preharvest interval (PHI) were studied and they were 2.71 and 10 days respectively.

Keywords: Spinetoram, residue, tomato, QuEChERS, HPLC

1.Introduction

Tomato (*Lycopersicon esculentum* L.) is one of the most important vegetable world wide. In Egypt, cultivated area with tomato plants represented about 34 % of the total area for vegetable crops. The annual production of tomato is estimated to be more than seven million tons from cultivated area about 223 thousand hectares regarding to (FAO stat 2012). The tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is a devastating pest of tomato plants. Following detection in the Spanish tomato-growing area at the end of 2006, the pest spread quickly to other European and northern African countries. It is an oligophagous pest that attacks solanaceous plants (EPPO, 2005, Pereyra and Sanchez, 2006). Recently, *T. absoluta* has also become a serious threat to tomato production in the Mediterranean region including Egypt (Seplyarsky, et al 2010). The larvae attack tomato plants during all growth stages, producing large galleries in their leaves, burrowing stalks, apical buds and green and ripe fruits. If no control measures are taken, then the pest can cause up to 80–100% yield losses on tomato crop.

Spinetoram is a multicomponent tetracyclic macrolide developed to control of lepidopterous larvae, leaf miners and thrips on different crops in many countries (Malhat, 2013). A very limited data have been reported about the degradation and residue of spinetoram on tomato since it is a relatively new insecticide. Analysis of spinetoram in different food matrices was reported by LC-MS/MMS (Kamel et al, 2010) and HPLC-DAD (Liu et al. 2010).

This study aimed to evaluate the dissipation of spinetoram in / on tomato fruit using HPLC with QuEChERS method.

2.Materials & Methods

Spinetoram is a fermentation product derived from the actinomycete bacterium *Saccharopolyspora spinosa*. Spinetoram consists of two related active ingredients, XDE-175-J and XDE- 175-L with ratio 3:1 as appeared at Fig.:1.

2.1.Reagent and chemicals:

The spinetoram analytical standard (purity ≥ 98%) was supplied by Dow agro sciences. All organic solvents employed throughout this study were HPLC grade and purchased from Merck (Darmsstadt, Germany). The HPLC system was an Agilent 1260 series attached to a photodiode array detector. Millipore – Q System was used for water purification. Primary secondary amine (PSA, 40 µm Bondesil) sorbents was purchased from Supelco (USA). Anhydrous magnesium sulfate was analytical grade purchased from Merck Ltd,. Sodium chloride and triethyl amine (TEA) was analytical grade purchased from El-Naser Pharmaceutical chemical Co., Egypt. Anhydrous magnesium sulfate and sodium chloride were activated by heating at 400°C for 4 hour in the oven before use and kept in the desiccators.

2.2.Field trial:

Tomato cultivar, GS12 was cultivated in a complete randomized block design at El-Kharaga District, New Valley Governorate, Egypt in the mid of February 2014. The cultivar was cultivated in four replicates (each one was 100 m²) and consisting of six tomato rows. Common agricultural and fertilization practices were used and no pesticides spray were applied to the test plots prior to or during this experiment.

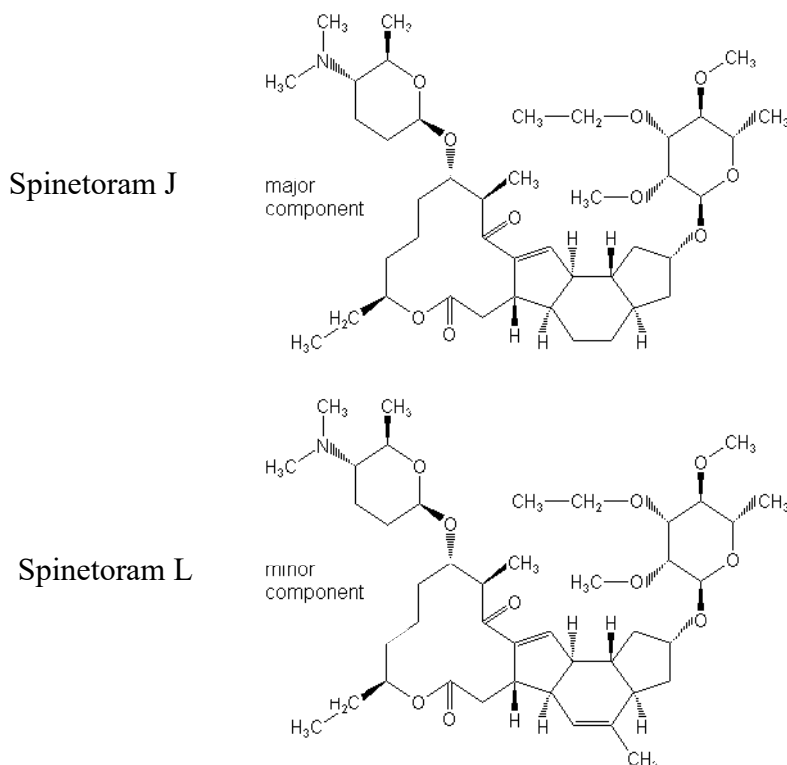


Fig. 1: Chemical structure of Spinetoram (J&L)

The commercial formulation of spinetoram (Radiant 12% SC) with the recommended dose (84 ml / hectare) was applied during the color stage of tomato fruits in mid May. The control plots were left untreated. Sampling was performed by randomly collecting from the field of the experimental plots according to the **FAO / WHO (1986)** recommendation. The fruit samples of treated and untreated plots were taken after (one hour after application or initial time), 1, 2, 7, 10 and 15 days from application. Random samples of 1 kg were collected from each replicate and packed in polyethylene bags. The samples transferred from the field in an ice box and kept in fridge until delivered to the laboratory. There was no rainfall in any time during the experimental period.

2.3. Analytical methods:

2.3.1. Sample preparation and extraction:

The extraction, cleaning and persistence analysis was carried out by triplicate. For residue analysis, tomato samples were homogenized for 5 min. at high speed in laboratory homogenizer and extracted. The homogenate of each sample was done according to the procedure described and modified by **Lehotay *et al.* 2010**. Where three representative samples of 10 gm were taken. Samples were then placed into polyethylene 50 ml centrifuge tube and analyzed immediately; 10 ml

acetonitrile (1% TEA) was added, and the mixture was vortexed for 2 min. After that, 4 gm of anhydrous magnesium, 1 gm of sodium chloride were added, then extract by shaking vigorously on vortex for 2 min and centrifuged for 10 min at 5000 rpm. An aliquot of 1 ml was transferred from supernatant to a new clean 50 ml centrifuge tube and cleaned by dispersive solid-phase extraction with 50 mg of PSA and 300 mg of magnesium sulfate. Afterwards, centrifugation was carried out at 5000 rpm for 5 min. Aliquot of 2 ml of the supernatant were taken and filtered through a 0.2 µm PTEE filter (Millipore, USA). The sample were then ready for the final analysis, the HPLC analysis was performed with an Agilent 1260 HPLC system (USA), with auto sampler injector, quaternary pump, thermostat compartment for the column and photodiode array detector. The chromatographic column was Zorbax C18 XDB (150 mm x 4.6 mm, 5 mm film thickness). The column was kept at room temperature. Flow rate of mobile phase (acetonitrile / methanol / ammonium acetate = 45+45+10; V/V/V) was 0.8 ml / min and the injection volume was 20 µl. Detection wavelength for detection of spinetoram was set at 246 nm. The residues in treated samples were identified by comparing the retention times (RTs) of the sample peaks with the (RTs)

of the injected standards. The recovery experiments were carried out on fresh untreated tomato fruits by fortifying the sample (10gm) in five replicates with spinetoram at three fortified levels (0.1, 0.5 and 1.0 mg kg⁻¹).

2.3.2. Calculation of half-life periods:

The half-life time ($t_{1/2}$) of spinetoram was calculated mathematically according to **Moye et. al (1987)** The degradation kinetics of the spinetoram residues were determined by comparing residue concentration against elapsed time after application and equation of best curve fit with maximum coefficients of determination (R^2) was determined. For dissipation of targeted insecticide in tomato fruits, exponential relationship was found to be applicable corresponding to the general first - order kinetics equation. ($C_t = C_0 e^{-kt}$) where, C_t = represents the concentration of the pesticide residue at the time of t , C_0 represent the initial deposits after application and k is the degradation rate constant in days⁻¹. The half-life ($t_{1/2}$) is recognized as the time required for the pesticide residue level to fall to half of the initial residue level after application and was calculated from the k value of each experiment, $t_{1/2} = \ln 2/k$.

3. Results & Discussion

To ensure the quality of the insecticide residue results, the performance of the method was evaluated considering different validation parameters that include the following items: the calibration curves of all of the compounds in pure solvent and matrix were obtained by plotting the peak area against the concentration of corresponding calibration standard at seven calibration levels ranging between 10 and 200 mg Kg⁻¹ (**Abd-Alrahman and Almaz, 2012**). For the preparation of calibration curves, spinetoram standard was either diluted with pure acetonitrile or with blank matrix extracts in series (three calibration point) from 0.1 to 1 µg ml⁻¹ (**Soliman, 2015**). In order to estimate the efficiency of the method, a recovery experiment was carried out by fortifying untreated samples with analytical grade spinetoram standard at the rate of 0.1, 0.5 and 1.0 mg kg⁻¹. Each fortification level was replicated five times. Extraction of untreated samples was performed as mention before. Results of the recovery are shown in Table1.

Table1: Recovery and relative standard deviation (RSD) for spinetoram on / in tomato fruit at various fortification levels.

Fortified level (mg / kg ⁻¹) n= 5	Recovery (%)	RSD (%)
0.1	88.81	7.5
0.5	92.39	3.4
1.0	95.41	5.3

n= number of replicates

From the above mentioned data in Table 1, the recoveries were ranged between 88.81% and 95.41% with associated RSD values ranged from 3.4 %, to 7.5 %. These results are considered to be highly satisfactory for the purpose of pesticide residue analysis and they are compatible with the European Union criteria which provide the average recoveries in the range 70 - 120 % with corresponding RSD less or equal 20% Document No. (**SANCO/ 12495/2011**). The limit of quantification (LOQ) of the method was defined as the lowest spiking level for which the validation criteria were satisfied and it was equal 0.1 mg kg⁻¹. Excellent linearity with the coefficient of determination (R^2) > 0.99 was achieved for the studied insecticide when using standard in the extract of tomato (matrix-matched standard).

The degradation of spinetoram in tomato fruits under New valley, Egypt climatic conditions has not previously explored systematically. The dissipation trends of spinetoram in tomato fruit were shown in Table 2.

Spinetoram dissipated rapidly after application. The residue of spinetoram in tomato samples after one hour of treatment was 1.087 mg/kg. Then the residues amount decreased to 0.809 mg/kg, in tomato fruit within the first 24 h after application. Following that period, spinetoram residues in/on tomato fruit decreased to 0.511, 0.148, 0.099, 0.07 and 0.04 mg/kg, at 3, 5, 7, 10 and 15 days after treatment, respectively. Samples taken 21 days after treatment contained no detectable amount of spinetoram (below the quantification limit 0.03 mg/kg) in tomato fruit. The half-life of spinetoram calculated in tomato fruit treated at recommended dose was 2.71-day (Table 2). The dissipation of the pesticide residues in/on crops depends on environmental condition, type of application, plant species, dosage, and interval between application, the relation between the treated surface and its weight and living state of the plant surface, in addition to harvest time (**Cabras et al. 1990 and Khay et al. 2008**), while the FAO/WHO has not established maximum residue limits (MRLs) for spinetoram, European Union MRL for spinetoram in tomato is 0.06 mg/kg. It can be concluded that the pre-harvest interval (PHI) of spinetoram on tomato was 10 days after the last treatment. This result is in agree with (**Malhat, 2013**). The degradation of the pesticide residues in /on crops depend on various factors, including environmental condition, chemical formulation, type of application, plant varieties, the interval between applications, the relation between the treated surface and its weight and state of the plant and also the harvest time (**Gennari et al. 1985; Cabras et al. 1989 and 1990; and Khay et al. 2008**).

The dissipation of spinetoram in/on tomato fruits with time shown in fig.2. The dissipation process of spinetoram in tomato fruits follows the first-order kinetics reactions.

As illustrated from the figure, the degradation of spinetoram residues followed the first order kinetics thereby showing varying dissipation through the 15 days period with half-life of 2.71 days.

European Union has established maximum residue limit (MRL) of in tomato as 0.06 mg kg⁻¹ (Codex, 2009) this can be concluded the PHI of spinetoram on tomato was 11 day after the last application. Regarding to the results obtained from the current study and the relevant residue regulation, spinetoram residue levels on / in tomato fruit will be acceptable when applied to control *Tuta absoluta* in Egypt according to short half-life time in tomato plants and also low final residue on fruits which was below the limit of detection (LOD).

Table 2. Residue of spinetoram on / in tomato fruits.

Time (Day after application)	Tomato fruit
Residues ±SD (mg kg ⁻¹)	% loss
Intial time *	1.087±0.969 0.00
1	0.809±0.158 25.57
3	0.511±0.080 53.08
5	0.148±0.010 86.38
7	0.099±0.022 90.89
10	0.07±0.001 93.56
15	0.04±0.001 96.32
21	ND
t _{1/2} (Day)	2.71
MRL (mg/kg ⁻¹ .)	0.06
PHI (Day)	10

PHI: Pre-harvest interval.

SD: Standard deviation.

t_{1/2} = ln 2/k.

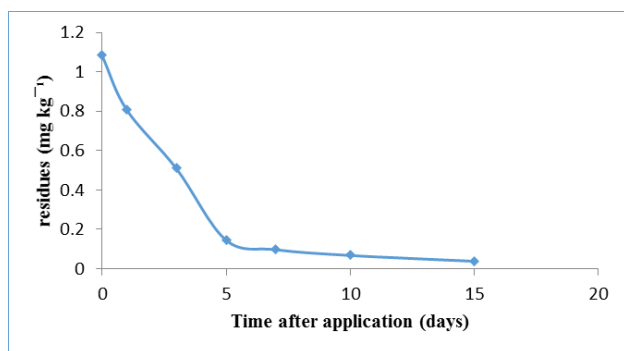


Fig. 2. Dissipation of spinetoram in/on tomato fruit with time.

Reference

Abd-Alrahman, S. H. and Almaz, M. M. (2012). Degradation of Propamocarb-hydrochloride in tomatoes, potatoes and

cucumber using HPLC–DAD and QuEChERS methodology. Bull. Environ. Contam. Toxicol. 89: 302-305.

Cabras, P.; Melion, M.; Cabitza, F and Cubeddu, M. (1989) Pesticide residues in the lettuce. 2. Influence of formulation. J. Agric. Food Chem. 37:1405-1407.

Cabras, P.; Spanedda, L.; Cabitza, F.; Cubeddu, M.; Martini, M. and Brandolini, G. (1990). Pirimicarb and its metabolites residues in lettuce. Influence of cultural environment. J. Agric. Food Chem. 38:879:882.

Codex Alimentarius commission (2009) codex maximum residue limits for pesticides. <http://apps.fao.org/faostat/collections?version=ext&hasbulk=0&subset=foodQuality>. FAO/WHO

EPPO. (2005). EPPO datasheets on quarantine pests: *Tuta absoluta*. EPPO Bulletin, 35: 434-435.

FAO stat (2012). Food and agricultural commodities production (country rank in the world by commodity). <http://faostat.fao.org/site/339/default.aspx>

FAO/WHO(1986). Recommended methods of sampling for determination of pesticide residues, vol. VIII, 2nd edn, pt VI.

Gennari, M.; Zannini, E.; Cingenti, A.; Bicchi, C.D.; Amato, A. Taccheo, M.; Spessotto, C.; DePaoli, M. and Flori, P. (1985). Vinclozolin decay on different vines in four different Italian area. J. Agric. Food Chem. 33:1232 – 1237.

Spessotto, C.; De Paoli, M. and Flori, P. (1985). Vinclozolin decay on different vines in four different Italian area. J. Agric. Food Chem. 33: 1232 – 1237.

Kamel, A.; Qian, Y.; Kolbe, E. and Safford, C. (2010). Development and validation of a multiresidue method for the determination of nionicotinoid and macrocyclic lactone pesticide residues in milk, fruits, and vegetables by ultra-performance liquid. J. of AOAC Int. 93(2):389-99

Khay, S.; Choie, J. and Abd El-Aty, M. (2008). Dissipation behavior of lufenuron, benzophenlurea insecticide in/on Chni cabbage applied by foliar spraying under Greenhouse condition. Bull. Environ. Contam. toxicol. 81: 369 – 372.

Lehotay, S.J.; Son, K.A. and Kwon, H. (2010). Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in fruits and vegetables. J. Chromatography A, 1217 (2010) 2548–2560

Liu, X.; Abd El-Aty, A.; Park, J.; Park, J.; Cho, S.; Shin, H. and Shim, J. (2010).

Determination of spinetoram in leafy vegetable crops using liquid chromatography and confirmation via tandem mass spectrometry. Biomed Chromatogr 25:1099–1106

Malhat F M (2013). Simultaneous determination of spinetoram residues in tomato by high Performance Liquid Chromatography combined with QuEChERS method. Bull. Environ. Contam. Toxicol. 90: 222 – 226.

Moye, H. A.; Malagodi M. H.; Yoh J.; Leibee G. I.; Ku C. C. and Wislocki P. G. (1987). Residues of avermectin B1a in rotational crops and soils following soil treatment with (C14) avermectin B1a. J. Agric. Food Chem., 35:859-864.

Pereyra, P. C. and Sanchez, N. E. (2006). Effect of two solanaceous plants on developmental and population parameters of the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Neotropical Entomology, 35(5): 671-676.

Seplyarsky, V.; Weiss, M. and Haberman, A. (2010). *Tuta absoluta* Povolny (Lepidoptera: Gelechiidae), a new invasive species in Israel. Phytoparasitica 38:445–446.

Soliman, H. M.A. (2015). Effect of the climatic changes on the persistence of some pesticide residues on and in tomato fruits. PhD. Thesis Benha University.119pp.