Dissipation rate of different commercial formulations of pyriproxyfen applied to orange fruits

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Abstract: Several commercial pyriproxyfen formulations lose their potency after a single application. The 10% EC and 10% EW of pyriproxyfen were determined using an improved QuEChERS technique and HPLC with DAD. The pyriproxyfen detection wavelength was set at 270 nm, and the mobile phase flow rate was set at 1 ml/min (acetonitrile/water = 70/30, v/v). At fortification levels of 0.01 and 1.0 mg/kg, pyriproxyfen recoveries on orange were reported to be 92.8%. Orange's LOQ was determined to be 0.1 g/kg. Pyriproxyfen residue diffused below the codex after 14 days, and the EU maximum residual limit (MRL) was between 0.6 and 0.5. According to the Codex and EU MRL values, the half-life (t 0.5) and PHI for pyriproxyfen 10% EC and 10% EW, respectively, are (13.8, 13.78, and 14.2, 14.2) days and (10.3, 9.6, and 9.5, 9.5) days. These results might aid in the proper and secure application of pyriproxyfen to orange fruits.

Keywords: Dissipation, pyriproxyfen, formulation, Orange, QuEChERS.

1.INTRODUCTION

In the period from 2015 to 2019, Egypt recorded yearly exports of vegetables and fruits to 27 countries in the European Union, totaling (133.428, 133.622, 161.134, 164.715, and 210.457 tons, respectively) **(GOEIC, 2020 and Abd El-Rahman, 2020).** Orange is one of the most widely consumed citrus (***Citrus L., Rutaceae***) fruits and plants worldwide, with a total production of 7,541,337 tones **(Farag *et al.*, 2020).** Almost 50 pesticides are recommended for use in Egypt to battle a variety of pests, diseases, and weeds, which makes citrus production, need the use of a huge number of pesticides. **(APC, 2020).** Due to the extensive and indiscriminate pesticide spraying on citrus plants, pesticide residues may have accumulated at levels above those maximum residue values (MRLs).

When these substances are applied in accordance with good agricultural practices, the maximum allowable residue of pesticides is not exceeded, but in the correct application may leave harmful residues that pose potential health and environmental risks. Pesticides are subject to government legislation and international organizations for the use of pesticides, including the maximum permissible residue of pesticides. These substances have been proven toxic, teratogenic, and carcinogenic. **(Fenoll *et al.*, 2009).**

With its pesticide action against flies, mosquitoes, and cockroaches—all of which pose a threat to public health—pyriproxyfen (CASS record number 95737-68-1) is a commonly used insect growth regulator. Pyriproxyfen has been used in vegetable and fruit farming to control scale, whitefly, almond worm, aphids, and cut worms. In South Africa and the Mediterranean basin, citrus crops are treated with the insecticide pyriproxyfen. The World Health Organization's Pesticides Assessment System has also evaluated pyriproxyfen for its ability to control vectors. **(WHO, 2008).**

Pyriproxyfen is one of the PPPs that is extremely effective and widely used in a range of fruits and fruiting vegetables, and it is one of the insecticides that the Egyptian Agricultural Pesticide Committee (APC) has allowed in order for citrus fruit to be safe (APC). The current study assesses the effects of two commercial Pyriproxyfen formulations (EC and EW) on oranges in Egyptian field settings and computes residual behaviour, pre-harvest intervals, and half-lives.

2.MATERIALS AND METHODS

2.1.Chemicals and reagents

Doctor Ehrenstorfer produced a Pyriproxyfen certified reference standard with a purity of over 99 % (Augsburg, Germany). Merck provided HPLC grade acetonitrile and methanol, as well as extra pure ammonium acetate, disodium hydrogen citrate sesquihydrate (Extra pure), trisodium citrate dihydrate (Extra pure), acetic acid, magnesium sulphate anhydrous fine powder, and sodium chloride. Supelco supplied bulk primary secondary amine (PSA) sorbent (Bondesil -PSA, 40 m).

2.2.Standard solutions

The pyriproxyfen standard stock solution was produced in acetonitrile at 100 g/ml and stored at 4ºC in the refrigerator. The working standard solutions (0.1, 0.5, 1.25, 2.5, and 5 mg/ml) were made by diluting the primary concentration of a standard stock solution in acetonitrile through a sequence of dilutions. Meanwhile, a standard solution equivalent to a matrix was created using the same titration strengths and an orange extract devoid of pesticide residues added to each dilute solution. Meanwhile, all of the completely produced solutions were kept at 4 degrees Celsius in the refrigerator. **(SANCO/12495, 2011).**

2.3.Pesticides used

The Pesticides tested are listed in Table (1) by Trade name, common chemical name, the manufacturer, and the recommended dose citrus treatment rate.

Table 1: List of pesticides and their rates of application used on citrus

Trade name	Common name	Application Rate(the Dose)	The manufacturer
Proximo 10% EC	pyriproxyfen	50 cm^3/100L	Industrias Afrasa S.A-Spain
Brophy 10% EC		50 cm^3/100L	Shanghai Shengnong Pesticide Co., Ltd. - China
Admiral 10% EW		50 cm^3/100L	Sumitomo Chemical Ltd-Japan
Acaro 10 % EW		50 cm^3/100L	Agri Sciences Tarim Ve Ilac Urunlerisan Ve Tic. Sti-Turkiy

2.4.Design of the field trial

Fifty citrus trees were selected from the Qalyubia governorate; these plants were severely infested with citrus scale insects *Coccoidea* and had not received pesticide treatment. As part of the experimental design, whole blocks were used. Using a backpack-motorized sprayer with an adjustable nozzle size of 1 mm, pyriproxyfen was applied using commercial formulations of Proximo 10% EC, Brophy 10% EC, Admiral 10% EW, and Acaro 10% EW. The manufacturer's recommended dose of 50 cm3 per 100 L1 of water was used to apply the pesticide. A control sample was taken at each sampling time. Samples were taken an hour after application (initial), 1, 3, 7, 10, 15 and 21 days later. Primary samples were obtained at random from the experimental plots according to Codex Alimentarius standard residue analysis techniques (**CODEX-Alimentarius, 1993)** and combined to a single laboratory sample (~2 kg) per plot. Samples were packed in ventilated polyethylene bags and immediately brought to the analytical laboratory, which was chopped and stored deep-frozen (−18ºC) prior to homogenization.

2.5.Analytical methods

2.5.1.Sample preparation

The orange samples were homogenized in a food processor (Thermomix; Vorwerk) and 10 g of the homogenate of each sample were placed into 50-mL centrifuge tube.

2.5.2.Sample extraction and clean up

10 ml acetonitrile (containing 1% acetic acid) was added to the orange sample (10 g), the screw cap was closed, and the tube was violently agitated for 1 minute using a vortex mixer at maximum speed. The material was then vortexed for 30 seconds after adding 1 g sodium chloride and 4 g anhydrous magnesium sulphate. The extracts were centrifuged at 3800 rpm and 4 ºC for 5 minutes. An aliquot of 4 mL was transferred from the supernatant to a new clean 15-mL centrifuge tube and cleaned up with 100 mg PSA, 20 mg GCB, and 600 mg MgSO4 using dispersive solid-phase extraction. The material was vortexed for another minute before centrifugation as described previously. The supernatant was then obtained in 2 mL increments, filtered using a 0.22-lm PTFE filter (Millipore, Billerica, MA), and transferred to a glass vial for HPLC-DAD analysis. **(Anastassiades *et al.*, 2003).**

2.5.3.HPLC determination

An Agilent 1100 Series HPLC system with quaternary pump, column thermostat compartment, and photodiode array detector was used for the HPLC study. Zorbax C18 XDB (250 mm 4.6 mm, 5 lm) was used as the chromatographic column. The temperature of the column was kept at room temperature. The mobile phase flow rate was 1 ml/min, and the injection volume was 20 µL (acetonitrile/water = 70/30, v/v). 270 nm was selected as the detection wavelength for pyriproxyfen. The residues in the real samples were tentatively identified by comparing the sample peaks' retention times (RTs) to the injected standards' RTs. At a retention time of 3.551 minutes, pyriproxyfen was eluted.

2.5.4.Calculation model

Evaluated the degradation of trend pyriproxyfen in orange using first-order kinetics. In accordance with previous reports, the dissipation kinetics equation was obtained as follows: $Ct=C0\ e{-}kt$, where Ct (µg/kg) and C0 (µg/kg) is the compound concentration at time t (day) and the initial concentration, respectively; and k (days−1) is the dissipation rate. The half-life (DT_{50}) was obtained with the following formula: DT_{50}= (ln 2)/k (**Fu *et al.*, 2017; Payá *et al.*, 2007).**

3.RESULTS AND DISCUSSION

Untreated orange samples were used to test the method's selectivity. The lack of any signal during the pyriproxyfen retention period suggested that no matrix chemicals were present, which might lead to a false positive result. In the examined range (0 to 10 g ml-1), the pyriproxyfen calibration curve demonstrated good linearity and a significant correlation between concentrations and peak area (r2≥ 0.999). The amount of pyriproxyfen recovered from orange was 92.8 %. Repeatability studies represented as RSD were used to investigate precision. Pyriproxyfen was found to have acceptable accuracy. For all three levels tested, the repeatability was less than 6%. Similarly, when looking at the matrix effect, there was a general trend towards greater RSD values at low spiking doses. Instrumental LOD with a S/N of 3:1 and LOQ with a S/N of 10:1 were developed (5 and 0.1 mg kg-1).Table 1 shows the mean residue levels of pyriproxyfen for each application determined from three sub samples across the sampling period. After applying the recommended dose of 50 cm3 100 L-1 water for orange throughout the study period, residue levels of pyriproxyfen were determined to be below the MRL established by the Codex Committee (0.5 mg kg-1) and the EU (0.6 mg kg-1). **(FAO/WHO, 2006).**

The primary samples obtained at the time of the first sampling 1 hour after the pesticide application had the greatest residual levels of the chemical pyriproxyfen. The samples treated with Proximo 10% EC and Brophy 10% EC had the greatest mean initial concentration of Pyriproxyfen residue (5.29 and 4.96 mg kg-1), followed by Admiral 10% EW and Acaro 10 % EW (4.2 and 4.08 mg kg-1) respectively. Pyriproxyfen residue levels decreased in the next period, reaching (4.91, 4.62 and 3.19, 2.97) mg kg-1 for Proximo 10% EC and Brophy 10% EC, respectively. Admiral 10% EW and Acaro 10 % EW were reaching (3.85, 3.67 and 2.21, 1.99) mg kg-1, respectively. All samples were collected after 21 days were free of any detectable of pyriproxyfen residues.

Proximo 10% EC, Brophy 10% EC , Admiral 10% EW and Acaro 10 % EW had varied half-lives ($t_{0.5}$) were (4.8 ,3.6, 2.9 and 2.9) days, respectively. PHI values according to Codex and EU MRL values of (13.8, 13.78, 10.3 and 9.6) days and (14.2, 14.2, 9.5 and 9.2) days, respectively. Other findings from the research **(Singh *et al.*, 2006)**. The initial deposit was 29.31 mg gk-1, which swiftly dissipated to 94.0 % dissipation in 10 days and 99.7 % dissipation in 15 days. However, according to a study by Beouwer, pesticide deposit dissipation is a complex process that is influenced by a variety of environmental factors such as temperature, relative humidity, and UV irradiation, metabolism and translocation (pesticide penetration and plant growth), application technique, and pesticide formulation. **(Brouwer *et al.*, 1997; Katag., 2004; Abd-Alrahman1 and Osama, 2012).**

Table 2. Pyriproxyfen mean concentration, half-live ($t_{1/2}$) and pre-harvest interval (PHI) on oranges

Time (days)	Proximo 10% EC		Brophy 10% EC		Admiral 10% EW		Acaro 10 % EW	
	Conc. (mg/kg)	Loss (%)	Conc. (mg/kg)	Loss (%)	Conc. (mg/kg)	Loss (%)	Conc. (mg/kg)	Loss (%)
0	5.29	00	4.96	00	4.2	00	4.08	00
1	4.91	7.18	4.62	6.85	3.85	8.33	3.67	10.05
3	3.19	39.69	2.97	40.12	2.21	47.38	1.99	51.23
7	2.03	61.62	1.83	63.10	1.11	73.57	1.00	75.49
10	0.97	81.66	0.87	82.46	0.53	87.38	0.48	88.24
15	0.41	92.24	0.4	91.94	0.37	91.19	0.33	91.91
21	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
t ½ days	4.8		3.6		2.9		2.9	
MRL (mg/kg) codex*	0.6							
MRL (mg/kg) EU**	0.5							
PHI (days)	13.8*	14.2**	13.78*	14.2**	10.3*	9.5**	9.6*	9.2**

Codex*:http://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/pestres/pesticide-detail/en/?p_id=200
Eu:**https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=pesticide.residue.CurrentMRL&language=EN

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معدلات التخفيف للمستحضرات التجارية المختلفة البيربروكسيفين المطبق على ثمار البرتقال

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الملخص العربى:

بعد التطبيق الفردي للعديد من مستحضرات البيربروكسيفين التجارية ، قدرت متبقيات البيربروكسيفين .باستخدام طريقة كاتيشير و جهازHPLC المزود بكاشف DAD ، تم تقدير متبقيات للبيربروكسيفين 10 ٪ فى صورتي المستحضر EC و . EW تم ضبط ظروف التحليل عند 1 مل / دقيقة أسيتونيتريل / ماء 30 / 70 = ، حجم / حجم)، وتم ضبط الطول الموجي للكشف عن البيربروكسيفين عند 270 نانومتر

وسجل متوسط معدل الاسترجاع البيربروكسيفين على البرتقال بنسبة 92.8 ٪ عند جرعات تدعيم تبلغ 0.01 و 1.0 مجم / كجم .بعد 14يومًا ، لم تظهر مخلفات البيربروكسيفين .وكان الحد الأقصى المتبقي للكودكس و الاتحاد الأوروبي هي (٠,٦ و ٠,٥ملجم /كجم) على الترتيب سجلت فترة نصف العمرلصورة المستحضر EC هي(٤,٨ و ٣,٦) يوم بينما في حالة المستحضر EW (٢,٩ و٢,٩) يوم ، على التوالي و سجلت قيمة فترة ما قبل الحصاد البيربروكسيفين 10 ٪ EC و البيربروكسيفين 10 ٪ EW ، على التوالي ، وفقًا لقيم كودكس و البالغة (١٣,٨و١٣,٧٨ و١٠,٣ و ٩,٦) يوم بينما طبقا لقيم الحدود القصوى الاوروبى (١٤,٢ و١٤,٢ و٩,٥ و٩,٢) يوم .قد تساعد هذه النتائج في التطبيق الصحيح والآمن البيربروكسيفين على ثمار البرتقال.