Research Article

ELISA, a feasible technique to monitor organophosphate, carbamate, and pyrethroid residues in local vegetables. Cuban case study

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Abstract

Organophosphates, carbamates, and pyrethroids are among the most commonly used pesticides worldwide. However, these pesticides are toxic not only to insects but also to other non-targets such as animals, including humans. The increasing public concern in recent years about possible health risks due to pesticide residues in the diet has influenced the strategies for crop protection. Over the years, researchers have relied on several analytical methods. The importance of enzyme-linked immune sorbent assays (ELISA) for pesticide analysis has increased over the past decades. This study was conducted to assess ELISA as a rapid, economical, and safe analytical procedure as an alternative prior to chromatographic techniques for monitoring the residues of these target pesticides in local Cuban vegetables. A colorimetric ELISA test kit was used to detect organophosphates and carbamates directly, while the analysis of pyrethroid was performed using paramagnetic particles attached to antibodies specifically to detect pyrethroids. To confirm the positive results, the samples were also analyzed by chromatography. With the use of the ELISA kits, it was possible to determine the presence of organophosphates, carbamates, and pyrethroid residues in the collected samples. The ELISA kits tested showed quantification capacity at values below the detection limit of the chromatographic techniques used. Linear relationships between the quantified values obtained by the chromatographic technique and results obtained through the pyrethroid ELISA test kits were observed. The developed ELISA exhibited high accuracy and is ideally suited as a fast, high-throughput, and low-cost screening test for organophosphates, carbamates, and pyrethroid residues to monitor and control these residue levels in the Cuban agriculture context.

Keywords Plant protection products · Acephate · Dimethoate · Immunoassay assessment · Sancti Spíritus

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1 Introduction

Fruit and vegetables can be carriers of pesticide residues if they are treated with pesticides. To lower the residue, good agricultural practices and respecting the as low as reasonably achievable “ALARA” principle are recommended. Organophosphates (OP), carbamates (CPs), and pyrethroids are among the most commonly used pesticides worldwide due to their broad biological activity and low bioaccumulation potential [1]. However, these pesticides are toxic not only to insects but also to other animals, such as amphibians, birds, and mammals, including humans. Some of these pesticides affect the human nerve system, inducing impulse transmission-inducing neurologic toxicity, chronic neurodevelopmental disorder, possible dysfunction of the immune, reproductive, and endocrine system or cancer [1, 2].

Exposure to OP, CPs, and pyrethroids can occur through the ingestion of contaminated food or water. The crop protection strategy has been updated in recent years with an emphasis on food quality and safety due to public concern about potential health risks from pesticide residues in food [3]. The widespread concern for the health of society led to strict regulation with maximum limits for pesticide residues in food commodities, potable and drinking water, soil, and general environmental media [1, 2, 4].

Cuba is not an exception to the use of OP, CPs, and pyrethroids, being these the families of insecticides more used in the control of plagues and diseases [5, 6]. As a result, some journalists reported and public expressed concerns about possible health risks due to pesticide residues, mainly in fresh crops (e.g. vegetables: tomato, sweet pepper, and cucumber). Since that, the government, together with the phytosanitary and human sanitary department, started to search for an analytical procedure to control and monitor pesticide residues [7, 8].

Over the years, technicians and researchers have relied on several analytical methods, such as gas chromatography (GC) and liquid chromatography (LC) for the detection, separation, and quantification of pesticide residues in different matrices [1]. However, it is not always convenient to use such detection tools due to their high cost, expensive instruments, long analysis duration, complex sample pretreatment, and the requirement of skilled labor [9]. Especially in developing countries like Cuba, with economic limitations hampering the use of chromatographic techniques. A group of authors cited by Kumar et al. [2] declares that fortunately, the need for simplified and portable detection techniques can be met through the use of biosensors, immunosensors, chemosensors, or electrochemical sensors.

The enzyme-linked immune sorbent assay (ELISA) technique may allow easy, rapid, low-cost, safe monitoring of a massive number of samples [1]. It offers remarkable advantages over chromatographic techniques in the detection of pesticides, biological toxins, pathogens, and drug residues [2, 9]. ELISA has been proven to be a low-cost, sensitive tool suitable for high throughput analysis, designed to monitor food contamination [10], mainly in terms of fast response, specificity, low detection limits, and most attractively, cost-effectiveness [2, 11]. Watanabe et al. [12] refer to its applicability as an analytical method for a simple and quick inspection of pesticide residues in agricultural products before shipment.

Abraxis Life Technologies™ provides field and lab-based ELISA testing kits for several pesticides tested in various matrices listed in the National Environmental Methods Index. Numerous articles have proven the suitability of Abraxis pesticide kits, especially for the analysis of glyphosate [13–15]. For OP and CPs, a colorimetric assay ELISA screen kit (Microtiter Plate: 96 Test) was purchased from Abraxis Inc. Ideal for consumers, dealers, and logistic enterprises, as it is more visual and intuitive than chromatographic or spectroscopic methods [16]. The color of the sample solutions can be observed with the naked eye for qualitative determination or analyzed through digital images for quantification [17].

The analysis of pyrethroid was performed using the Abraxis Pyrethroid Assay kit (using paramagnetic particles attached with antibodies specific to pyrethroids). A competitive reaction for antibody binding sites on magnetic particles develops between pyrethroids (present in the sample) and the permethrin analog marked with an enzyme (the enzyme conjugate) [18]. Since the labeled permethrin (conjugate) is in competition with the unlabeled pyrethroids (sample) for the antibody sites, the color developed is inversely proportional to the concentration of pyrethroids in the sample. The magnetic enzyme-linked immunosorbent assay has attracted interest recently [9]. Sullivan et al. (2007) showed the feasibility of detecting chlorpyrifos using a commercial magnetic particle-based ELISA kit [2].

Having in mind that there is no previous history in Cuba of using the ELISA test to monitor and control pesticide residues, the results obtained in this study could serve as a basis for the integration of ELISA in pesticide residue monitoring procedures in Cuba. The study intends to evaluate the suitability of the ELISA technique for the monitoring and control of residues of organophosphates, carbamates, and pyrethroids insecticides in vegetables locally grown in Cuba, prior to chromatographic techniques (gas chromatography or liquid chromatography).

2 Materials and methods

2.1 Materials and instruments

Of the analytical chromatography grade, OP, CPs, and pyrethroid standards were purchased from Sigma-Aldrich (Belgium). Sigma-Aldrich also supplied sodium hydrogencitrate sesquihydrate ($C_6H_6Na_2O_7 \cdot \frac{1}{2}H_2O$) 99%, sodium chloride (NaCl) > 99%, sodium citrate tribasic dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) > 99% and the highest analytical purity pesticides standards needed in the study. Magnesium sulfate anhydrous ($MgSO_4$) came from Merck (Belgium). HPLC grade acetonitrile (ACN) was supplied by VWR (BDH PROLABO, Belgium) and n-hexane > 99% assay was obtained from Chem-Lab (Belgium).

2.2 Sample collection

Thirty-four samples from three agricultural areas in Sancti Spíritus (Banao, Cabaiguán, and La Quinta) were collected during March 2019. The samples consisted of tomatoes (19), sweet pepper (10), and cucumber (5). The ELISA tests were performed 2 days after collection in the Laboratories of the Centre for Energy and Industrial Process Studies of the University of Sancti Spíritus, Cuba. Samples were blended to homogenize and distributed over two different centrifuge tubes. In the first one, used for the ELISA assay, 2 g of the homogenized sample was mix with 2 g of methanol by handshaking as the ELISA kit suggests. Then the tubes were centrifuged at 10,000 rpm for 5 min. A subsample volume (around 1 mL) for the ELISA assay was taken from the clear supernatant. In the second tube, 10 g of the homogenized sample was kept frozen at $-20^\circ C$ until further analysis in the Laboratory of Crop Protection Chemistry of Ghent University by the QuEChERS “quick, easy, cheap, effective, rugged, and safe” method. Gas chromatography with Electron Capture Detector (GC-ECD) was used for the pyrethroids analysis and ultra-performance liquid chromatography-tandem mass spectrometer (UPLC-MS/MS) for the OP, CPs.

2.3 Elisa

The analysis was developed following the corresponding procedure explained in each test kit. A Vortex-Genie 2 (VWR International; Edmonton) was used and a magnetic separator rack was supplied by Abraxis. The final reading was performed with a spectrophotometer (Rayleigh VIS723G, China) at 405 and 450 nm.

2.4 ELISA control

Pyrethroids

The Abraxis pyrethroid assay kit provides screening results. As with any analytical technique samples requiring regulatory action should be confirmed by an alternative method, so results will be confirmed by a chromatographic technique in the lab.

Therefore, a four-point calibration curve (0.75, 2.5, 5.0, and $15 \mu g L^{-1}$), as proposed in the manual guide of the Abraxis pyrethroid assay kit, was used. Different standards were prepared: permethrin (pyrethroid) standards in water (control) as well as in the matrices (tomato, sweet pepper, and cucumber). Also, a midrange standard (positive control: $3.0 \mu g L^{-1}$) was developed to check the accuracy of the curves, as the manual recommended. Subsequently, the unknown samples were tested. Three replicate analyses were conducted and average values were reported. As a manual guide dictates the %B/Bo was reported as the mean absorbance value for each standard divided by the mean absorbance value for the Diluent/Zero Standard. By plotting the %B/Bo for each standard on the vertical Logit (Y) axis versus the corresponding pyrethroid concentration on the horizontal Ln (X) axis a standard curve was constructed. Controls and samples will then yield levels in $\mu g L^{-1}$ of pyrethroid by interpolation in the standard curve or using equation [18]. Sample results were multiplied by a factor of 2 to account for the initial 1:1 dilution of the sample with methanol. The assay has an estimated minimum detectable concentration, based on a 90% B/Bo, of $0.75 \mu g L^{-1}$ (for permethrin), which was more than adequate according to the level of pyrethroids expected in the fresh vegetables ($10.0 \mu g L^{-1}$). The Abraxis test brochure mentions that an average of 98% recovery was obtained from five-times diluted groundwater samples spiked with various levels of permethrin. In addition, the cross-reactivity for various Pyrethroid analogs (shown in Table 1) can be expressed as the least detectable dose (LDD) which is estimated at the dose required for 50% absorbance inhibition (50% B/Bo) [18].

Table 1 Limit of detection and inhibition percentage of pyrethroids commonly use in Cuban, using the Abraxis ELISA kit

Compound	LDD ($\mu g L^{-1}$)	50% B/Bo ($\mu g L^{-1}$)
Permethrin	0.75	4.25
Cypermethrin	4.75	100
Lambda (λ) cyhalothrin	9.20	90
Bifenthrin	13.50	150
Cyfluthrin	220	3400
Tetramethrin	> 1000	> 10,000

The following compounds demonstrated no reactivity in the Abraxis Pyrethroid Assay at concentrations up to $1000 \mu\text{g L}^{-1}$: alachlor, atrazine, benomyl, captan, carbaryl, carbendazim, 2,4-D, 1,3-dichloropropene, metolachlor, metribuzin, picloram, and thiabendazole.

Organophosphate and carbamate

The assay is a modification of Ellman's method, based on a modification of his acetylcholinesterase (ACh-E) enzyme inhibition. The presence of organophosphate or carbamate pesticides in a sample will inhibit ACh-E, reducing or eliminating color formation, depending on the concentration [19].

The accuracy of OP and CPs pesticides will differ depending on their ability to inhibit the enzyme. Besides, manual guidance states that the test can be used for quantitative testing if there is only a single OP or CP in the sample [19]. The estimated minimum limit of detection based on a 20% inhibition of the color developed for OP or CP in 50% methanol is shown in Table 2. Abraxis plate assays suggest diazinon at $5.0 \mu\text{g L}^{-1}$ as the positive control. When samples showed a percent inhibition lower than 20%, they were regarded as negative, and vice versa. With the relation between the absorbance values obtained for the negative control (distilled water) and the positive control, a linear calculation is performed to predict the OP or CPs values of the analyzed samples. The test is invalid and must be repeated for accurate results if the negative control does not produce a yellow color when the assay is completed. The Abraxis OP and CP kit thus provide first semi-quantitative screening results. Should be confirmed by an alternative method all samples that require a regulatory action [19].

The OP and CPs plate assay allows for the analysis of 46 samples in duplicate determination. Less than 1 mL of sample is required, and the test is performed in less than 1 h. Abraxis evaluated the precision in the OP and CPs assay, by spiking three pools with diazinon at $0.5 \mu\text{g L}^{-1}$, $1.0 \mu\text{g L}^{-1}$, and $1.5 \mu\text{g L}^{-1}$ in duplicate and running

each assay five times. Between assays, a variation coefficient of 7.9%, 3.4%, and 4.5% was obtained for $0.5 \mu\text{g L}^{-1}$, $1.0 \mu\text{g L}^{-1}$, and $1.5 \mu\text{g L}^{-1}$, respectively, and a precision of 3.9%, 3.1% and 3.5% within the assay.

The analytical sensitivity of the kit can be affected if samples contain gross particulate matter. It is required to filter before the analysis to a particle size equal to or less than $0.2 \mu\text{m}$. If performing the assay outdoors, direct sunlight should be avoided. Also, pigmented samples may obscure color, potentially causing interferences. Therefore, the negative control should be prepared in a similar matrix and analyzed with the pigmented samples [19].

2.5 Comparative analysis

The QuEChERS method was used as a simple analytical extraction method for the detection of multiple pesticide residues in fruit, vegetables, and other matrices [20]. In addition, tomato, sweet pepper, and cucumber blank samples were spiked first at $15 \mu\text{g L}^{-1}$ of permethrin (pyrethroid) and then $5 \mu\text{g L}^{-1}$ diazinon (organophosphate). Both positive controls of the Abraxis kits to confirm the kit performance. Extracts were analyzed by GC-ECD using Agilent Technologies 6890 N, and a Waters ACQUITY UPLC-MS/MS.

A detailed description of the analytical method and equipment conditions

To the 10 g of vegetables of a homogenous made sample weighed and conserved in the centrifuge tubes (50 mL), 15 mL of acetonitrile (ACN) was added and shaken. The following salts were added to each sample to remove co-extracted contaminants: 1.5 g NaCl, 1.5 g $\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 2\text{H}_2\text{O}$, 0.750 g $\text{C}_6\text{H}_6\text{Na}_2\text{O}_7 \cdot \frac{1}{2}\text{H}_2\text{O}$ and 6.0 g MgSO_4 . Sample, solvent, and salts were mixed and then separated shaking for 5 min by 300 rpm and centrifuged 5 min at 10,000 rpm. The solvent exchange is different for the LC-MS/MS and GC-ECD samples. For the LC-MS/MS samples 1 mL of the upper layer was sampled and added to a volumetric flask of 10 mL. 9 mL Milli-Q water was added to obtain a total volume of 10 mL. A subsample of $\pm 1.5 \text{ mL}$ was pipetted in an LC-MS/MS vial. For the GC-ECD samples, 5 mL of the upper layer was sampled to an evaporation bowl. The solvent (ACN) was evaporated in the Rotary evaporator and 5 mL of n-hexane was added to the bowls to recover the analyte. A subsample of $\pm 1.5 \text{ mL}$ was pipetted in a GC-ECD vial.

Ultra-performance liquid chromatography operating conditions

Table 2 Limit of detection of organophosphates and carbamates commonly used in Cuban for the Abraxis ELISA kit

Organophosphate	$\mu\text{g L}^{-1}$	Carbamate	$\mu\text{g L}^{-1}$
Chlorpyrifos methyl	0.4	Carbaryl	206
Chlorpyrifos ethyl	0.5	Carbofuran	0.9
Diazinon	0.6		
Dichlorvos	0.5		
Ethion	0.6		
Malathion	1.2		
Parathion	0.8		

A Waters ACQUITY UPLC™, equipped with a quaternary pump and triple quadrupole system with electrospray ionization (Waters Xevo® TQD) to perform sample analyses was used. The separation column, an Acquity UPLC BEH C18, 130Å (1.7 µm 2.1 mm 50 mm) was kept at 40 °C. 10 µL per sample was automatic injected. The mobile phase components were (A) Milli-Q water with 0.1% formic acid and (B) ACN with 0.1% formic acid. A flow rate of 0.4 mL min⁻¹ of 98% mobile phase A for 0.25 min was used as a gradient set. From 0.25 to 7 min, a linear gradient was used to 98% mobile phase B, held for 1 min. Then a linear gradient was used to 98% mobile phase A and held for 1 min. The capillary needle was maintained at +2 kV, curtain gas (N₂) at 7 bars, and temperature 500 °C. The AIs were monitored and quantified using multiple reactions monitoring (MRM). The MS/MS-transitions, ionization mode, cone voltage, and collision energy are given in supplementary material.

Gas chromatography with electron capture detection

As previously described by De Maegt et al. [21] in her work, an Agilent Technologies 6890N gas chromatograph equipped with an Agilent Technologies 7683 Series autosampler injector, coupled to an electron capture detector (GC-ECD) was used. Separation was performed on a HP-5MS (5% phenyl methyl siloxane) capillary column (30 m 0.25 mm 0.25 µm). As operating conditions, the column initially set at a temperature of 60 °C and then the oven temperature was increased at a rate of 20 °C min⁻¹ to 150 °C. Furthermore, it was increased at a rate of 15 °C min⁻¹ to 250 °C, held for 2 min at 250 °C, followed by an increase at a rate of 30 °C min⁻¹ to 270 °C and held constant for 10 min at 270 °C. Thereafter, it was increased at a rate of 30 °C min⁻¹ to 280 °C and finally, it was held at 280 °C for 11 min. Injector and detector temperatures were maintained at 200 °C and 250 °C, respectively. Helium was

used as a carrier gas at a flow rate of 1.1 mL min⁻¹ and the injections were made in the split mode with a split ratio of 52.7:1 [21].

3 Results and discussion

3.1 ELISA test for pyrethroids

Figure 1 shows the calibration curves for permethrin developed in water (control) and in the tested matrices (tomato, sweet pepper, and cucumber) using the pyrethroid ELISA kit. As the manual of the kit recommends, dilutions of clean vegetable juices (1:1 in methanol) should be used as reference instead of clear water for the spectrophotometric measurements.

Statistically significant correspondence between the calibration curves in the matrices and the control curve in water was found. The slope from calibration curves in the tomato, sweet pepper, and cucumber matrices were between the confidence limits (upper: -0.12311 and lower: -0.17749) of the control slope curve, for n-2 degrees of freedom to the desired probability of 95%. The concentration measured in the positive control, i.e. 3.46 µg L⁻¹, corresponded relatively well with the expected concentration (3.0 µg L⁻¹), with 115% recovery. The calibration curve (n=4) showed 4.0% of the coefficient of variation. Xu et al. [22] also compared four ELISA kit brands for the analysis of organophosphates, carbamates, and pyrethroids. Their best results were obtained with the kits provided by Abraxis, with a recovery percentage and coefficient of variation very similar to the values obtained in our study. Figure 1 shows the percentages of inhibition of absorbance concerning the negative control from 0.75 to 15.0 µg L⁻¹ of permethrin in the evaluated matrixes. Based on those results, calibration curves from each matrix were used for

Fig. 1 Calibration curves in clear water and clear juice from food matrices (tomato, sweet pepper, cucumber) evaluated during the pyrethroid (permethrin) test of the ELISA kit

the calculation of the corresponding concentration in the evaluated samples.

3.2 Comparison of results from ELISA with GC-ECD and UPLC–MS/MS

The accuracy of the ELISA results for pyrethroids analysis could not be evaluated by GC-ECD analysis, as the GC-ECD equipment used has a limit of quantification (LOQ) (considered here as the lowest point in the calibration curve) for permethrin of $100.0 \mu\text{g L}^{-1}$, around seven times higher than the spiked concentration evaluated ($15.0 \mu\text{g L}^{-1}$) in the ELISA test. From the group of synthetic pyrethroids that are analyzed in the laboratory, permethrin is one of the least sensitive (ten times less than cypermethrin, bifenthrin, and others). From the group of pyrethroids analyzed by GC-ECD, permethrin should be the active ingredient with the highest response factor (injected concentration/peak area) [23]. The ELISA test thus has a clear added value for the monitoring and control of pyrethroid residues in the vegetables studied due to its lower detection limit.

Permethrin is widely used for hygienic control in sanitary in Cuba and together with other pyrethroids in phytosanitary control. Sometimes these pesticides are used incorrectly. They reach water bodies and/or remain as residue in certain crops [24–26]. Unlike in the EU, where the use of permethrin is not allowed, in Cuba permethrin and other pyrethroids can still be used. If Cuba is considering the export of some of these vegetables or fruits to Europe, it should meet the EU Maximum residual levels (MRL), set for permethrin at concentrations below $50.0 \mu\text{g kg}^{-1}$. The ELISA test can meet these requirements.

The accuracy of ELISA results obtained for OP and CPs were evaluated by UPLC–MS/MS analysis. Table 3 shows the results of the positive ELISA control samples in the different matrices, evaluated by liquid chromatography analysis. Percentages of inhibition were obtained in the ELISA tests, which could be verified by chromatography. An average concentration of $5.69 \mu\text{g L}^{-1}$ for the different matrices evaluated, a 114% recovery, and a 3% coefficient of variation may be obtained with the LC analysis respect to the ELISA test. Similar results were reported in Chinese

and Indian studies [27–29]. In this way, the accuracy of the ELISA test for OP and CPs could be proven.

3.3 Screened samples

As can be observed in Table 4, with the use of ELISA a higher amount of samples with OP, CPs, and pyrethroids residues were quantified compared to the chromatography techniques that only detected them. Luo et al. (2017) also mention that the immunoassay was capable to detect ethyl carbamates in a large number of samples. A small signal was obtained in the chromatograms of several samples at the retention time of thiodicarb, methiocarb, acephate, dimethoate, oxamyl, cypermethrin among others, but the ratio signal/noise was too low to consider this signal for detection. In addition, two samples of tomato in OP and CPs test with concentrations from ELISA of $0.0025 \text{ mg kg}^{-1}$ and $0.0023 \text{ mg kg}^{-1}$ (results can be a cumulative residue of various OP and CPs with individual concentrations below the detection limit of the chromatographic technique used), as well as in two cucumber samples from ELISA pyrethroids with the concentration of $0.0026 \text{ mg kg}^{-1}$ and $0.0042 \text{ mg kg}^{-1}$ no corresponding signals were found in the applied chromatographic techniques. In the brochure of the Abraxis kit for pyrethroids, OP, and CPs, it is indicated that the limit of quantification for some pesticide residues (Tables 1, 2) is lower than that of the chromatographic technique used. Although these values can be quantified by ELISA, they can be only detected by the chromatographic technique used in this study. All detected values were below their MRL.

Figure 2 shows the relationship between the values obtained by chromatographic techniques and the ELISA values presented in Table 2. Linear relationships were found. Carbaryl values (left graph) show a slope of 1.0089 with R^2 of 0.9983. The left graph (carbaryl) shows only two points because only in two of the samples analyzed by LC it was possible to quantify its concentration and compare it with the results obtained in the ELISA analysis.

Also, cypermethrin (right graph) shows a slope of 1.1088 with R^2 of 0.9986. Additionally, a satisfactory Pearson correlation $r = 0.999$ ($p < 0.001$) was found. Other authors also obtained well-correlated results between

Table 3 Recovery values of the OP positive controls evaluated by LC–MS/MS

Matrices	Positive control spiked ($\mu\text{g L}^{-1}$)	ELISA inhibition (%) at 405 nm	LC values ($\mu\text{g L}^{-1}$)	Mean	Recovery (%)	SD	CoV (%)
Tomato	5.00	74	5.54	5.69	114	0.0002	3
Sweet pepper		78	5.62				
Cucumber		80	5.91				

LC liquid chromatography, SD standard deviation, CoV coefficient of variation

Table 4 Organophosphate, carbamate and pyrethroids residues detected in the screened samples using the ELISA test and chromatographic methods

Samples	Chromatographic				ELISA (mg kg ⁻¹)	Chromatographic		ELISA (mg kg ⁻¹)
	Carbamate (CP)	Organophosphate (OP)				pyrethroid		
	Carbaryl (mg kg ⁻¹)	Pro-fenofos (mg kg ⁻¹)	Acephate (mg kg ⁻¹)	Dimethoate (mg kg ⁻¹)		Cypermethrin (mg kg ⁻¹)	Tau-fluvalinate (mg kg ⁻¹)	
Sweet pepper	0.0038				0.0038		< LOQ	0.0019
Cucumber	*	< LOQ		< LOQ	0.0049			0.0026
Cucumber		< LOQ		< LOQ	0.0034			0.0042
Tomato					0.0025			
Tomato			< LOQ	< LOQ	0.0034	< LOQ		0.0089
Tomato				< LOQ	0.0052	< LOQ		0.0053
Tomato			< LOQ	< LOQ	0.0030			
Tomato			< LOQ	< LOQ	0.0035			
Tomato			< LOQ		0.0030			
Tomato				< LOQ	0.0027			
Tomato					0.0023			
Tomato				< LOQ	0.0025			
Tomato			< LOQ		0.0026			
Tomato				< LOQ	0.0043			
Sweet pepper		< LOQ			0.0027			
Sweet pepper	0.0045			< LOQ	0.0046		< LOQ	0.0102
Sweet pepper		< LOQ			0.0030			
Cucumber		< LOQ			0.0025			
Cucumber						< LOQ		0.0145
Cucumber				< LOQ	0.0024	0.0158		0.0174
Sweet pepper						0.0379		0.0422
Sweet pepper						0.0230		0.0270
Sweet pepper						0.0502		0.0550
Sweet pepper						0.0636		0.0705
EU/Cuban MRL (mg kg ⁻¹)	0.01/5.0	0.01/-	0.01/-	0.01/-		0.50/0.2	0.10/-	
Tomato	0.01/-	0.01/5.0	0.01/-	0.01/-		0.20/0.07	0.05/-	
Cucumber	0.01/5.0		0.01/-	0.01/0.5		0.50/0.1	0.01/-	
Sweet peppers								
LOQ (mg L ⁻¹)	0.001	0.001	0.001	0.001		0.01	0.01	

*Blank space means < LOD

ELISA and chromatographic techniques in their sample analysis, suggesting good accuracy and reproducibility of the ELISA methods [10, 30, 31].

If a brief elementary economic analysis is performed, it is observed that only the cost of the salts and solvents used in the extraction procedure (QuEChERS) prior to the chromatography analysis represents 50% of the cost of the Abraxis kit for OP and CPs and the 34% of the Pyrethroid Kit, for the same number of samples. Besides, a chromatograph has approximately a cost 100 times higher than the cost of a reader to conduct the ELISA analysis. It should also be considered that the maintenance costs, technical support, and spare parts required for a chromatograph are

much higher than those required for a colorimetric reader. Additionally, ELISA tests have an additional advantage over chromatographic analyses in that such tests can be developed in the field for qualitative screening.

Thus ELISA receives important attention, especially for residues of OP, CPs, and pyrethroids, several of which are prohibited or of restricted use, mainly in the EU, where they received a default MRLs value. The developed ELISA exhibited good accuracy, is ideally suited as a fast, high-throughput and low-cost screening test for OP and CPs and pyrethroids residues to monitor and control the level of such residues at local (municipal) levels. However, the authors do not rule out the benefits of analysis by

Fig. 2 Analysis of the linear relationship for the cross quantified values between ELISA test and chromatographic alternatives (left: carbaryl in sweet pepper, right: cypermethrin in sweet pepper (last four-point) and cucumber) shown in Table 2

chromatographic techniques, which provide a general spectrum of all residues present in case of a legal requirement. So chromatographic analytical capacities should still be developed at regional levels in Cuba, where tests can be developed and results can be validated, leaving the more economic ELISA tests for routine monitoring and control.

3.4 General pesticide residue detected

Table 5 shows, in addition to the pesticides of interest in the study (OP, CPs, and pyrethroids), other pesticide residues detected by chromatography in the samples analyzed. As can be observed, residues of the other 13 different active ingredients (AIs) were detected. Fungicide was the most common group with nine AIs measured in the samples. Three of them were triazoles. Future research will focus on monitoring fungicide residues by ELISA tests. Leyva Morales et al. [32], also identified fungicides as the group with the highest frequency of use in northwestern Mexico; Wahid et al. [33] cite fungicides as the second pesticide group imported after herbicides in Suriname. Additionally, EFSA [34] reported in the 2015 annual report of pesticides in food, fungicides as the most frequent pesticides with concentrations equal to or greater than the LOQ found. Two insecticides (neonicotinoids) and two herbicides completed the list of the residues detected.

For seven of the samples analyzed, AI residues showing the same mode of action, like the neonicotinoids (imidacloprid and thiamethoxam) and the triazoles (difenoconazole, propiconazole, and tebuconazole), were found. Farmers should be alerted on the hazard and risk of developing resistance to pests and diseases if AIs with the same mode of action are used on the same crop in

one season [35–37]. From 13 of the AIs detected in the collected samples, seven (fenpropimorph, chlorothalonil, thiamethoxam, carbendazim, propiconazole, ametryn, and alachlor) are forbidden for use in the EU. If the carbamate and organophosphates from Table 4 are also included, this number increases to 11 [38].

It should also be noted that MRL values are currently absent in the Cuban legislation [39] for 14 of the 19 AIs detected. This hinders their control and monitoring. The AIs detected without Cuban MRLs include those banned from use in the EU: acephate, ametryn, fenpropimorph, thiamethoxam, carbendazim, propiconazole, dimethoate, and alachlor. Alachlor had value even higher than the European MRL.

Although the presence of AI residues that are prohibited in the EU persists, the number of these has decreased, based on results reported in previous studies published in journals that were reviewed. In samples of vegetables collected in the period 2016–2018, residues of non-authorized use pesticides [40] like methamidophos, parathion and parathion methyl, and lindane were additionally found. Therefore, the presence of the residues mentioned in the samples collected between 2016 and 2018 meant a violation of the established laws and/or due to possible illegal activities, because after 2016, their use is not allowed. Positive is the fact that the group of AIs (mainly OP and CPs) already banned in several countries, mainly in the EU and the United States, which is still included in the current list of authorized pesticides in Cuba, is gradually decreasing. That is part of a program for the reduction of synthetic pesticides with high toxicity, promoted by the government of Cuba in support of national and international environmental laws. It aims to guarantee

Table 5 Pesticide residues quantified (mg kg^{-1}) by GC-ECD and UPLC-MS/MS in the collected samples

Sample #	Fenproph-morph mg kg^{-1}	Imidaclo- prid	Chlorotha- lonil	Difenocona- zole	Thiameth- oxam	Carbenda- zim	Propicon- zole	Azox- ystrobin	Pyrimetha- nil	Tebucona- zole	Ametryn	Alachlor	Prochloraz
Sweet pep- per	< LOQ	0.019				0.014		0.002		0.002			
Cucumber *		0.002			0.010								
Cucumber		0.002		< LOQ	0.013								
Tomato	< LOQ		0.018		< LOQ	< LOQ							
Tomato	< LOQ												
Tomato			0.036						0.005				
Tomato	< LOQ		0.105										
Tomato	< LOQ	< LOQ											
Tomato	< LOQ		0.099						< LOQ				
Tomato	< LOQ		< LOQ										
Tomato	< LOQ		0.024			< LOQ							
Tomato	< LOQ		< LOQ					< LOQ					
Tomato	< LOQ	< LOQ											
Tomato	0.003	< LOQ											
Tomato	< LOQ		0.015										
Sweet pep- per		0.021				0.018		0.003		0.002			0.001
Sweet pep- per		0.002		< LOQ	0.010								
Sweet pep- per		< LOQ			0.003								
Sweet pep- per	< LOQ	0.002		0.002	0.010								
Cucumber		0.003									< LOQ	0.018	
Cucumber	< LOQ												
Sweet pep- per		0.020		0.005			0.005						
Sweet pep- per		0.020		0.004			0.004						
Sweet pep- per		0.020		0.005			0.005						
Sweet pep- per		0.019		0.006			0.007						

Table 5 (continued)

Sample #	Fenpropi- morph mg kg ⁻¹	Imidaclo- prid	Chlorotha- lonil	Difenocona- zole	Thiameth- oxam	Carbenda- zim	Propicon- zole	Azoxy- strobin	Pyrimetha- nil	Tebucona- zole	Ametryn	Alachlor	Prochloraz
EU/Cuban MRL (mg kg ⁻¹)	0.01/- 0.01/- 0.01/-	0.5/0.5 1.0/1.0 1.0/1.0	6.0/5.0 5.0/5.0 0.01/7.0	2.0/0.5 0.3/- 0.9/-	0.2/- 0.5/- 0.7/-	0.3/- 0.1/- 0.1/-	3.0/- 0.01/- 0.01/-	3.0/3.0 1.0/1.0 3.0/3.0	1.0/0.7 0.8/- 2.0/-	0.9/0.2 0.6/0.2 0.6/0.5	0.01/- 0.01/- 0.01/-	0.01/- 0.01/- 0.01/-	0.05/- 0.05/- 0.05/-
Tomato													
Cucumber													
Sweet pep- pers													
LOQ (mg kg ⁻¹)	0.001	0.001	0.01	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.01	0.001

*Blank space means < LOD

food safety without compromising human health and environmental protection [25, 41–43].

4 Conclusion

The study fulfilled its purpose, with the use of Abraxis ELISA kits, it was possible to detect the presence of the residues of certain groups of compounds of interest like organo-phosphates, carbamates, and pyrethroids in the collected samples. ELISA proved to be a reliable low-cost analytical procedure for fast detection, control, and monitoring of the presence of pesticide residues in tomatoes, sweet peppers, and cucumbers, before chromatographic techniques (gas or liquid chromatography). The ELISA kits tested showed the capacity for quantification at values below the detection limit of the chromatographic techniques used. However, further analysis to determine specific active ingredients and their quantity should still be done by chromatographic techniques, which also allows more pesticides to be analyzed. More than half of the total residues detected in the collected samples pointed towards the use of synthetic pesticides which are nowadays banned in the EU. In the Cuban agriculture context, ELISA can be used as a tool to assess pesticide usage as currently still a lot of carbamates, organo-phosphates, and pyrethroids are being used. Surveillance and control actions would be mainly focused on guaranteeing that the crops to be exported comply with international residue limits, as well as ensuring that the population does not ingest highly toxic pesticide residues.

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