Exploring matrix effects in liquid chromatography-tandem mass spectrometry determination of pesticide residues in tropical fruits

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Editor in Chief

Castellon, 26th January 2015

Dear Dr. Wise,

Please find enclosed our paper entitled **Exploring matrix effects in liquid chromatography-tandem mass spectrometry determination of pesticide residues in tropical fruits,** by A.M. Botero-Coy, J.M. Marín, R. Serrano, J.V. Sancho, F. Hernández, which we submit to Analytical and Bioanalytical Chemistry after revision following the referees' comments.

We have answered all the referees' comments (see separate document), and also made a revision of the English trying to simplify the text and to improve the readability of the manuscript.

We hope after this revision the paper can be considered acceptable for publication in Analytical and Bioanalytical Chemistry.

Yours sincerely

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Exploring matrix effects in liquid chromatography-tandem mass spectrometry determination of pesticide residues in tropical fruits

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ABSTRACT

Tropical fruits are being increasingly consumed around the world because of their appreciated characteristics, particularly their high nutritional value and distinctive taste, different to traditional fruits. Due to their introduction in international markets it is necessary to have a reliable analytical methodology available for the sensitive determination of pesticide residues in order to monitor the compliance of maximum residue limits (MRLs)From an analytical point of view, tropical fruits have generally been far less studied than other fruits frequently consumed in the European Union or USA, which are among the most important markets. In this work, LC-MS/MS-based methodology using triple quadrupole analyzer has been developed for the multi-residue determination of selected pesticides and metabolites in tropical fruits, which were selected among the most popular in Colombia, one of the most important suppliers of tropical fruits around the world. After selection of a QuEChERS (**Qu**ick, **E**asy, **Ch**eap, **E**ffective, **R**ugged and **S**afe)-based sample treatment, the study was focused on matrix effects evaluation, in order to find a simple way for their correction. Twelve different food matrices were selected to perform this study: the seven Colombian tropical fruits of highest value for domestic and international markets (uchuva, tamarillo, granadilla, gulupa, maracuya, papaya and pithaya), and five more matrices highly consumed in Colombia (lulo, carambolo, feijoa, mangostan and guayaba). Twenty compounds, including pesticides widely applied in tropical fruits pest control and several metabolites considered in residue definition, were used as model compounds in this work. Correction factors were used on the basis of calibration graphs obtained with standards in solvent and in matrix, and their usefulness was supported by validation of the method in all the matrices tested at 0.01 mg/kg and 0.1 mg/kg. The analysis of real-world samples revealed the presence of several target compounds that were identified by the acquisition of two MS/MS transitions, and by ion intensity ratio and retention time agreement.

KEYWORDS

Pesticide residue analysis; tropical fruits; matrix effects, LC-tandem MS, Colombia fruit matrices

1. INTRODUCTION

Tropical fruits are of great importance for the economy of several countries around the world, particularly in South America, Asia or Africa, where agricultural activities are mainly based on these types of crops. They are grown under special climatic conditions that give them particular nutritional and organoleptic characteristics. The demand for tropical fruits has increased in the last years because of their particular characteristics of taste, flavor and vitamin content (e.g Vitamin C), carotenes and antioxidant components [1]. Consequently, there is interest in developing and/or adapting analytical methodologies for the determination of pesticide residues in tropical fruits, in order to monitor the compliance of Maximum Residue Limits (MRL). Moreover, in many of these products, MRLs are set by default at a specific low value (i.e. the limit of determination of an analytical method developed for each pesticide in another (similar) food matrix) [2]. This is due to the lack of studies on residue trials performed in compliance with the principles of Good Laboratory Practices (GLP) directed towards registration of the product and the establishing of MRL. It seems clear that analytical methodologies are currently required for tropical fruits, in order to monitor the compliance of MRLs, but also to facilitate the performance of the analytical part of GLP studies to set-up new MRLs on the basis of new residue trails.

Colombia is one of the main suppliers of exotic fruits in the world. Among the main fruits exported are uchuva, tamarillo, tamarindo, granadilla, pithaya, gulupa and baby banana. The main destinations of these products are The Netherlands, Germany, France, Belgium and Spain. It is worth noting that Colombia is the world's first producer of uchuva . In 2012, the total export value of Colombian uchuva was USD 29.2 million, and it was the most important fruit in International trademark, followed by gulupa (USD 12 million), granadilla (USD 2.9 million), pithaya (USD 2 million), “tomate de árbol” or tamarillo (USD 1.3 million) and, to a lesser extent, maracuya and feijoa, giving a total of USD 48,6 million. During the first term of 2014, an increase of 14.5% was observed in tropical fruits exportation in relation to 2013 [3].

The use of Multi-Residue Methods (MRMs) is currently required in the field of Pesticide Residue Analysis (PRA) as the only realistic way to monitor a large number of pesticides in the great number of samples that are commonly analyzed in specialized laboratories. Most MRMs reported for fruits and vegetables in the last decade are based on the use of liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), which is considered the technique of choice for the majority of pesticides and metabolites. Its excellent sensitivity, selectivity and robustness, and its suitability for most pesticides currently used, of medium-high polarity and medium-low volatility, are

among the main reasons for its wide use [4-14]. However, little attention has been paid to tropical fruits, and there is a general lack of analytical methodology available for these types of food matrices. Some of the methods reported are based on gas chromatography with conventional detectors, and on LC-UV/VIS or fluorescence detectors [15-17], which required the confirmation of positive findings by MS. Papaya, mango and guava are among the most studied tropical matrices [15,16] [18-21].

The results on pesticide residues in a wide monitoring of fruit and vegetable samples from South America revealed the post-harvest fungicides thiabendazole and imazalil, and the insecticide chlorpyrifos as the pesticides most frequently detected [22]. Pesticides detected in tropical fruits, like papaya, mango and passion fruit, were chlorothalonil, dimethoate, thiacloprid, imidacloprid, methomyl, cypermetrin, lamda-cyhalotrin, propamocarb, and dithiocarbamates. Recently, a GC-MS multi-residue method based on the use of QuEChERS CEN (European Committee for Standardization) procedure has been developed for 50 pesticides in tropical fruits, and validated for tomato, tamarillo and goldenberries (*uchuva*). The method was applied to the analysis of samples collected from Antioquia (Colombia), and allowed an initial risk assessment, especially for tomatoes, where several pesticides such carbaryl, carbofuran, diazinon, dimethoate, endosulfan alpha, endosulfan beta and p,p´DDT were detected [23].

Taylor [24] considered matrix effects as the “Achilles heel” of LC-MS based methods. Previously to his paper and especially in the last decade, many articles have been reported dealing with matrix effects in LC-MS/MS methods for pesticide residues in environmental, biological and food matrices [6] [13] [25-35]. Different alternatives are normally applied to remove, minimize and/or correct this undesirable effect [26] [27]. The most popular are the use of matrix-matched standards calibration, the application of clean-up steps along the sample treatment, and the use of appropriate internal standards (commonly, isotope-labeled internal standards ILIS) [6] [26] [35] [36]. In theory, one of the most accurate approaches is standard additions, but unfortunately it increases the number of injections and requires to roughly knowing the analyte concentration in the sample to adjust the additions at the correct level. Moreover, ILIS are expensive and not always commercially available. Their use is rather frequent in single methods for specific pesticides, but not in MRMs where a high number of ILIS would be required. Other possibilities, such as optimization of chromatographic separation and/or MS measurements [26] [37] are less applicable in MRMs involving large numbers of compounds. It has been also reported the selection of a few representative matrices to prepare matrix-matched standards for all type of samples

analyzed, assuming that matrix effects are comparable between similar matrices [38]. Thus, Kmellar et al. classified the samples analyzed into three groups for preparation of calibration curves: tomato, representing commodities of high water content; pear for commodities of high sugar content; and orange for those of high acidic content [9]. Dilution of sample extracts can be also used to minimize matrix effects and to make different sample extracts more similar if the method has sufficient sensitivity, a fact that is being more common with the instrumentation available nowadays [32].

Matrix effects can lead to both ionization suppression and enhancement. This fact clearly affects quantification of analytes if not properly corrected.But matrix effects may also affect the identification of the compound detected, as this process is normally based on the acquisition of two SRM transitions (in tandem MS methods): one for quantification and the other for confirmation of the identity. Typically, the second transition is less intense than the first one due to the lower abundance of the product ion selected. Thus, strong ionization suppression may hamper the presence of the peak at the second transition, avoiding the confirmation of the compound at low concentrations. In addition, the presence of co-eluting matrix interferences sharing the ions used for quantification and/or confirmation may also affect the ion intensity ratio, hindering its compliance within the tolerances admitted [39] [40]. As a consequence, matrix effects need to be properly corrected; this being one of the most challenging tasks in LC-MS/MS based MRMs.

Different sample treatments have been developed for pesticide residue analysis in fruits and vegetables. Among them, the QuEChERS procedure has become the most popular, as illustrated by the high number of references from the first publication [41]. The original procedure was based on extraction with acetonitrile, separation of water from acetonitrile by addition of anhydrous $MgSO_4$ and NaCl, and subsequent clean-up using dispersive solid-phase extraction (d-SPE) with a primary secondary amine (PSA), which efficiently removes many polar interfering substances present in the matrix. From the original unbuffered version published in 2003 [41], different versions/modifications have been reported to improve its applicability to more and more pesticides, especially for pH-dependent pesticides, and more complex sample matrices [42-44]. The most popular accepted versions are the AOAC (Association of Official Agricultural Chemists) Official Method 2007, which uses acetate buffer [45], and the European Committee for Standarization (CEN) Standard Method EN 15662, which uses citrate buffering [46]. A combination of different sorbents can be used in d-SPE to improve the clean-up step. Thus, a mixture of three sorbents (C18, PSA and graphitized carbon black (GCB)) has been shown efficient for most analytes tested

[43]. Recently, a comparison of QuEChERS official methodologies has been made for the multi-residue determination of 33 pesticides in Colombian fruits by GC-MS using large volume injection [47]. The CEN method was preferred since acceptable recoveries were achieved for all analytes. The use of GCB in the clean-up step did not improve the results and it was found not to be much useful for clean-up purposes.

In this article, we have developed an analytical methodology for the LC-MS/MS residue determination of 20 compounds (including 6 metabolites) frequently applied for pest control in tropical fruits. Twelve tropical food matrices were selected among those of highest commercial value in Colombia. Their common and scientific names, taxonomic classification and inclusion in the EU group products for MRLs compliance [2] are included in **Figure 1**. 9 out of 12 products are included in fresh fruits group (miscellaneous fruits), while the remaining 3 belong to the solanaceae family and are included in vegetable fresh group (fruiting vegetables). Most of MRLs applied to the pesticides and food products studied in this work are set-up at default values of 0.01, 0.02 or 0.05 mg/kg, which correspond to the limit of determination/quantification of the analytical method (marked as (*) in **Table 1, Supplementary Information).** QuEChERS (CEN citrate version) was selected for sample extraction and clean-up, and LC-MS/MS with triple quadrupole was used for analysis.. Special attention was paid to matrix effects, trying to find a simple and generic solution for appropriate correction. The applicability of the method was tested by analyzing samples collected from local markets at Colombia and samples exported to Spain.

2. Experimental

2.1. Reagents and chemicals

Pesticide reference standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany). HPLC-grade methanol, HPLC-grade acetonitrile (ACN) and acetone for residue analysis, Magnesium sulfate, Sodium Chloride, Sodium hydrogencitrate sesquihydrate and Sodium Citrate were purchased from Scharlau (Barcelona, Spain). HPLC-grade water was obtained by purifying demineralized water in a Milli-Q Gradient A10 (Millipore, Bedford, MA, USA). Formic acid (HCOOH, 98 - 100%) and ammonium acetate (NH_4Ac, reagent grade) were supplied by Scharlau.

Stock standard solutions were prepared dissolving 50 mg, accurately weighted, in 100 mL of acetone obtaining a final concentration of around 500 mg/L. For LC-MS analysis, the stock solutions were diluted with acetonitrile to prepare individual

solutions of around 50 mg/L. From these, mixed solutions of 5 pesticides were prepared by diluting with acetonitrile to obtain a final concentration of 5 mg/L. Working mixed solutions of all pesticides were prepared from the 5 mg/L solutions by dilution with acetonitrile.

Mixed solutions of 1 mg/L and 0.1 mg/L in acetonitrile were used for sample fortification in recovery experiments.

In the clean-up step, two types of 2-mL microcentrifuge tubes for QuEChERS d-SPE were used, containing: 150 mg anhydrous $MgSO_4$, 25 mg PSA and 25 mg C18 (XE-29508); or 150 mg anhydrous $MgSO_4$ and 50 mg PSA (XE-29511) (Teknokroma, Barcelona, Spain).

2.2. Liquid chromatography/Mass Spectrometry

A Waters Alliance 2795 LC system (Waters, Milford, MA, USA) was interfaced to a Quattro micro triple quadrupole mass spectrometer (Waters) using an orthogonal Z-spray-electrospray interface. The LC separation was performed using Atlantis dC_{18} column (5µm, 2.1 x 100 mm; Waters) at a flow rate of 0.3 mL/min. The mobile phase used was water/ methanol (both 0.1mM NH_4Ac and 0.01% (2 mM) HCOOH) gradient, where the percentage of methanol changed as follows: 0 min, 5%; 1 min, 5%; 10 min, 90%; 13 min, 90%, 14.1min, 5%.

Drying gas as well as nebulising gas was nitrogen (Praxair, Valencia, Spain). The desolvation gas and cone gas flows were adjusted to 600 and 60 L/h, respectively. Infusion experiments were performed using the built-in syringe pump, directly connected to the interface. For operation in MS/MS mode, the collision gas was argon (99.995%; Praxair, Valencia, Spain) at a pressure of 2×10^{-3} mbar in the collision cell. Capillary voltage of 3.5 KV in positive mode was used.

The interface temperature was set to 350 ºC and the source temperature to 120 ºC. Dwell times of 0.1 s were chosen. Two solvent delays were selected to give an additional clean-up using the built-in divert valve controlled by the Masslynx v.4.1 software, the first one from 0 to 4.5 min and the second one from 15 to 17 min. The application manager TargetLynx was used to process the quantitative data obtained from calibration standards and from samples.

2.3 Samples

Samples used in this study were exported from Colombia to the European Union, specifically to Spain. They were acquired in Spanish markets and hypermarkets from

Barcelona and from Castellon. Then, they were transported to the laboratory and processed for analysis. All samples (commonly 6 individual pieces) were homogenised (pulp, small stones and peel). Stones were removed before triturating only in the case of mangostan, due to their larger size. In the case of uchuva, the calyx was also removed. This group of samples, acquired at Spain, were used for analysis and also as "blank" samples for validation of the method. Another group of samples were collected directly in Bogotá, where they were acquired in a local market. They were processed as indicated above and the triturated sample was stored in the freezer at <-18ºC. Later, they were transported to Spain were they arrived within a maximum period of time of 24 h. This second group of samples was used for analysis and, also to prepare quality controls (QCs) of the analytical procedure.

2.4. Recommended procedure

10 g of homogenized sample were accurately weighed (precision 0.1 mg) in a 50 mL polypropylene centrifuge tube. Extraction was carried out using 10mL acetonitrile, shaking by hand for 1 min. Then, 4 g Magnesium Sulfate, 1 g Sodium Chloride, 0.5 g Sodium Hydrogencitrate Sesquihydrate and 1 g Sodium Citrate were added and immediately shaken vigorously by hand to prevent formation of $MgSO_4$ agglomerates. The tube was centrifuged at 4600 rpm for 10 min.

For the cleanup step, 1 mL of the upper ACN extract was poured into a d-SPE tube containing 150 mg $MgSO_4$, 25 mg PSA and 25 mg C18. The tubes were shaken on a vortex for 30 s and centrifuged at 12000 rpm for 7 min. Then, 10 µL of the final ACN extract was directly injected into the LC system under the experimental conditions indicated in section 3.1. Quantification of samples was made by external calibration with standards in solvent by applying the correction factors obtained in this work (see section 3.3. matrix effects).

The scheme of the procedure applied in shown in **Figure 1 SI**.

2.5. Matrix effects evaluation

For evaluation of matrix effects, matrix-matched calibration was prepared for each matrix type by taking 450 µL of the blank sample extract and adding 50 µL of the corresponding standard in acetonitrile (between 25 and 5000 ng/mL), resulting in final concentrations between 2.5 and 500 ng/mL).

Matrix effect was evaluated by calculating the percentage of signal suppression or enhancement using equation:

$$Slopes\ difference = \frac{Matrix\ Calibration\ Slope - Direct\ Calibration\ Slope}{Direct\ Calibration\ Slope}\ X\ 100 \quad [1]$$

Then, correction factors were estimated for each sample matrix by using the following equation (for details see **Figure 2 SI**):

$$F = \frac{1}{1 + \frac{Slopes\ \ difference\ (\%)}{100}} \quad [2]$$

In analysis of samples, the concentration of the pesticide residue was obtained by multiplying the concentration obtained after application of direct calibration with standards in solvent by the corresponding correction factor (see section 3.3. matrix effects).

2.6. Validation study

Fortification of samples for recovery experiments was performed by delivering 1 mL of 0.1 mg/L or 1 mg/L standard mixture solutions in acetonitrile to 10 g homogenized blank sample in order to yield fortification levels of 0.01 mg/kg or 0.1 mg/kg, respectively. The fortified samples were left to stand for 1 h prior to extraction.

Validation of the method was based on European Union SANCO (Directorate-General for Health and Consumer Protection) guideline [39]. Precision (repeatability, in terms of % RSD) and accuracy (percentage recoveries) were estimated by recovery experiments at two fortification levels, 0.01 and 0.1 mg/kg (analyzed in quintuplicate). The limit of quantification (LOQ) objective was set as the lowest concentration that was validated in fortified samples with satisfactory precision (RSD≤ 20%) and recovery (between 70–120%).

The specificity of the method was evaluated using the quantitative transition (Q) by analysing a procedure blank, a processed blank sample, and a processed blank sample spiked at the LOQ level. The acceptance criteria was that both, procedure and sample blanks, did not present any relevant chromatographic peak at the transition selected (<30%).

The limit of detection (LOD), defined as the lowest analyte concentration that could be detected and differentiated from the sample blank, i.e. corresponding to a

signal-to-noise ratio of 3, was estimated from the chromatograms of sample extracts fortified at the lowest level tested (i.e. 10 ng/mL).

Confirmation of the identity of the compound in samples was carried out by acquisition of two MS/MS transitions and the compliance of the q/Q ratio between samples and reference standards with maximum tolerances of ± 30%. The agreement in retention time was also required, with maximum deviation of ±0.2 min [39].

3. RESULTS AND DISCUSSION

In a previous work, we developed analytical methodology for around 30 pesticides in seven tropical fruit matrices selected among the most important for the export market of Colombia: uchuva, maracuya, pithaya, tamarillo, gulupa, papaya and granadilla [38]. The LOQ objective at that work was 0.05 mg/kg, which was satisfactory for most pesticide/matrix combinations in terms of MRLs compliance. However, in a few cases the default MRL values are established in the present regulation at values lower than 0.05 mg/kg, commonly 0.01 and 0.02 mg/kg [2]. Also, metabolites are included in the residue definition for some pesticides. Therefore, the objective of the present work was to make a selection of pesticides commonly applied in Colombia and to update the analytical methodology in a higher number of tropical fruit matrices. The present work was focused only on those compounds from the previous list [38] that include metabolites in their residue definition (dimethoate, that includes its metabolite omethoate; thiamethoxam/clothianidin; carbofuran/3-hydroxy carbofuran; diuron/3,4-dichloroaniline; malathion/malaoxon; parathion methyl/paraoxon methyl). Two pesticides (benomyl and thiodicarb), that are applied in the field as precursors of carbendazim and methomyl respectively, were not considered in this work because of the unlikely presence of these compounds in the samples due to their conversion after application in the field and/or degradation along laboratory sample treatment to carbendazim and methomyl. Those compounds with MRLs default values for tropical fruits matrices below 0.05 mg/kg were also included in this work (dimethoate; picloram; carbofuran; clomazone; parathion methyl; malathion). Another three compounds were also added in relation to the previous work (imazalil, thiacloprid, thiabendazol) as they have been found in some tropical fruits [16] [22]. Altogether, 14 pesticides and 6 metabolites were selected to perform the present study **(Table 1).**

3.1. MS and chromatographic conditions

All spectra were obtained by infusion of 2.5 mg/L standard solutions in methanol/water (50:50, v/v) at a flow rate of 10 μL/min. The highest sensitivity was observed for all compounds in positive ESI. The full-scan spectrum showed the most abundant ions for each compound, which typically corresponded to the protonated molecule. Different cone voltages, between 5 and 50 V, were tested to optimize the abundance of the $[M+H]^+$ ion, selecting the values shown in **Table 1**.

The formation of sodium adducts (e,g., dimethoate, omethoate, picloram, paraoxon methyl, parathion methyl, malaoxon, 3,4 dicloroaniline, diuron, clomazone and chlorpyrifos), which are poorly fragmented and not much recommendable in MS/MS-based methods, was minimized by adding formic acid and/or ammonium acetate, favoring in this way the formation of the protonated molecule $[M+H]^+$, finally selected for all precursor ions.

MS/MS experiments were performed at different collision energies. Working under selected reaction monitoring (SRM) mode, the most sensitive transition (Q) was used for quantification purposes, while the second one was used for confirmation of the identity (q) **(Table 1).**

An analytical column Atlantis dC_{18} (5μm, 2.1x100 mm) was selected in this study following the good results obtained in the previous work [38]. In order to optimize the chromatographic conditions, a mixed standard solution with all pesticides at 50 ng/mL was used. First of all, MeOH and ACN were checked as organic solvents in the mobile phase. As the studied compounds were optimized in ESI positive mode, the presence of a protic solvent such as MeOH improved the sensitivity for all the compounds (with the exception of 3,4-dichloroaniline). Furthermore, the analytes' peak shapes were mostly better with MeOH than using ACN.

Due to the presence of omethoate, a rather polar compound, the initial percentage of organic phase (methanol) was fixed at 5% for better retention in the C_{18} chromatographic column employed. Although the extract injected into the LC-MS/MS (10 μL) containing 100% of organic solvent (acetonitrile), the peak shapes were acceptable. Just in the case of thiabendazole and carbendazim, band broadening was observed. The addition of mobile phase modifiers (HCOOH and NH_4Ac both in water and MeOH) improved peak shape and sensitivity for most of the studied compounds according to the MS infusion experiments carried out in the previous step. Thus, several percentages of ammonium acetate (0.05-1 mM) and formic acid (0.005-0.1%) were tested both in the aqueous and organic phases. The use of 0.1 mM of NH_4Ac and

0.01% of formic acid was selected as a compromise between satisfactory peak shape and sensitivity for all compounds,.

Finally, the chromatographic conditions selected were: an Atlantis dC_{18} column with MeOH:H_2O (both 0.1 mM NH_4Ac and 0.01% HCOOH) as mobile phase at a flow rate of 0.3 mL/min, with a gradient where the percentage of MeOH changed as follows: 0 min,5%; 1 min,5%; 10 min, 90%; 13 min, 90%; 14.1 min, 5%. Under these conditions, the compounds eluted as shown in **Table 1**, with retention times between 5.7 min (omethoate) and 13.3 min (chlorpyrifos). In order to achieve satisfactory number of points per chromatographic peak (at least 10), the two SRM transitions per compound were distributed in individual functions. Under the final conditions selected, matrix-matched standards at 50 ng/mL were also injected to test the chromatographic behavior of the analytes in the matrices tested. A similar behavior was observed in all tropical fruits in relation to retention times and peak shape.

3.2. Sample treatment

In this work, we applied the QuEChERS citrate-buffering version [46]. The d-SPE clean-up was made with a mixture of $MgSO_4$, PSA and C_{18}. A scheme of the procedure applied is shown in **Figure 1 SI.**

After application of the extraction step, two different clean-up systems were tested: a mixture of $MgSO_4$, PSA and C_{18} by one side, and $MgSO_4$ and PSA by other side. The addition of C_{18} together with the primary-secondary amine (PSA) in the d-SPE step has been reported to improve the cleanup for some samples, particularly those that contain lipids such as olives, and it does no harm in any case [44]. Although some chemists employ a freeze-out step to reduce lipid coextractives, C_{18} in d-SPE is faster and easier, and has been shown to work equally well in removing lipids, although freezing out also precipitates additional matrix components having limited solubility in QuEChERS extracts [48].

Not significant differences were found in recoveries and matrix effects among the two clean-up methods tested, although slightly better results were found for the mixture $MgSO_4$, PSA and C_{18}. Therefore, this was the approach used in this work. The results obtained for picloram were not satisfactory, as it could not be properly recovered after the QuEChERS procedure applied. Surely, the retention of this acidic analyte (pKa 2.3) in PSA material was the main reason of the low recoveries. This is in agreement with the literature, as low recoveries for this compound have been reported in food matrices [49] [50]. Degradation of picloram by amino or PSA sorbents has been also suggested

as the reason of the low recoveries consistently obtained when these columns are used with spiked extracts [50]. The low recoveries for acidic compounds when using PSA for clean-up has been widely reported [51].

3.3. Matrix effects

As stated in the Introduction, matrix effects are one of the main problems associated to LC-MS/(MS) methods. Among the different possibilities to minimize and/or compensate this undesirable effect, the most popular in MRMs is the use of matrix-matched standards calibration. It is also common to select a few representative matrices to prepare matrix-matched standards when performing routine analysis of large number of samples, assuming that matrix effects are comparable i between similar matrices [9] [29] [33] [34] [38] [44] [49].

In this work, a detailed study of matrix effects was made by comparison of standards prepared in solvent and in matrix, a common way to test matrix effects. The comparison of slopes obtained from calibration curves constructed in the presence of matrix and in pure solvent has been also used to evaluate signal suppression or enhancement [9] [29] [44] [52]. Both, ionization suppression and enhancement were observed depending on the analyte/matrix combination under study. As an alternative to the use of matrix-matched standards calibration for every matrix analyzed, we tested a simple way that avoids the preparation of matrix-matched standards every time that a set of samples needs to be analyzed. The approach consisted on preparing the calibration curves for every analyte in solvent and in the twelve tropical matrices studied to evaluate whether ionization suppression or enhancement took place from the slopes of the calibration graphs. The differences in slopes between calibration in solvent and in matrix were calculated according to equation **[1],** and the correction factors were estimated for every analyte in every matrix using equation **[2].** These correction factors can be applied in future analysis, allowing performing analysis without the need of preparing new calibrations in matrix, just using standards in solvent.

As illustrative example, **Figure 2** shows the differences in calibration graphs for several compounds investigated. From this figure, it is easy to appreciate the enhancement ionization for methomyl in several matrices, as, lulo, mangostan and granadilla (Figure 2 a), the absence of matrix effects for dimethoate (only slight enhancement observed for lulo) (Figure 2b), and the matrix suppression occurring in several matrices for thiacloprid (Figure 2c) and in most matrices for chlorpyrifos (Figure 2d).

The correction factors resulting from this experiment are summarized in **Figure 3**. We assumed that no relevant matrix effect occurred when differences in slopes between calibration in matrix and in solvent were up to ± 20%. Therefore, no correction was applied in those cases (uncolored boxes). Green/dark boxes refer to matrix enhancement effects (slope difference above 20%), and yellow/light boxes refer to matrix suppression effects (slope difference below -20%).

Omethoate and dimethoate were not much affected by matrix effects, together with diuron that only showed matrix suppression for mangostan. For several compounds, no signal suppression was observed for any of the matrices, and thus matrix effects, when present, led to only signal enhancement (methomyl, thiametoxam, 3-OH carbofuran, thiabendazol, imazalil, 3,4-dichloroaniline). It is worth to notice that imazalil and thiabendazol were affected by ionization enhancement in all matrices tested. The occurrence of important matrix effects for imazalil is in agreement with data reported by other authors [32].

On the contrary, for a few compounds only signal suppression was observed, although not in all matrices (paraoxon methyl, carbofuran, clomazone, parathion methyl, chlorpyrifos). Among these, parathion methyl and chlorpyrifos were affected by ionization suppression in nearly all matrices tested (11 out of 12, and 10 out of 12, respectively). Some other compounds were affected ion both ways, showing matrix suppression for some matrices and matrix enhancement for others (carbendazim, clothianidin, thiacloprid, malathion).

In relation to the matrix sample analyzed, some trends were observed. For example, guayaba, carambolo and tamarillo showed signal enhancement for almost all pesticides, while mangostan and feijoa led predominantly to signal suppression. The most difficult sample in terms of matrix effects was mangostan, where strong matrix effects were mostly observed.

The different behavior observed for compounds and food matrices under study showed that matrix effects were not homogeneous as a function of the chromatographic retention or the matrix analyzed, despite that some trends, as previously commented, were observed. Therefore, it seems not easy to predict the signal and extension of the matrix effects for each analyte/matrix combination.

3.4. Method validation

The usefulness of the approach used in this work was evaluated by calculating recoveries in fortified samples after applying the overall analytical procedure (i.e

process efficiency, which includes the extraction process recovery and the influence of matrix effects). Six matrices were selected and fortified (before extraction) at two concentrations, 0.01 and 0.1 mg/kg. Analyses were performed in quintuplicate, using calibration curves with standards in solvent that were introduced at the beginning and the end of each sequence of analysis of every matrix sample.

The process efficiency was calculated using the concentrations obtained by direct calibration (standards in solvent) and also after application of the corrections factors corresponding to the analyte/matrix combination under study (see Figure 3). Correction factors were only applied when matrix effects were significant (i.e. differences in slopes above ± 20%; green and yellow color boxes in Figure 3), simulating the procedure that would be applied in routine analysis.

Figure 4 shows the results obtained in this experiment for the six matrices evaluated at the low concentration level tested (0.01 mg/kg). In general, the correction was satisfactory for all matrices, leading to recoveries in the range 70-120%, and data were consistent at the two concentrations tested. It can be easily visualized the satisfactory correction for granadilla for several pesticides whose recoveries were out of the 70-120% range. However, after correction, recoveries reached the desired values for up to 6 pesticides that were out of tolerance before correction (thiamethoxan, clothianidin, thiabendazole, thiacloprid, paraoxon methyl, parathion methyl) (Figure 4a). In tamarillo, 4 pesticides that were out of tolerances were satisfactorily corrected by applying the correction factors (Figure 4b). The same occurred for 8 pesticides in uchuva, the matrix for which correction was more significant (Figure 4c); 5 pesticides in pithaya (Figure 4d); 5 in maracuya (Figure 4e) and 6 in gulupa (Figure 4f). Apart from a few cases where the correction did not seem sufficient, the general trend was satisfactory.. 3,4-dichloroaniline (metabolite of diuron) did consistently show recovery values below 70% (mostly between 40 and 60%) in four of the matrices tested (granadilla, tamarillo, uchuva, pithaya). This might be explained because this analyte may form strong bonds with common substances present in vegetable matrices and/or due to partial degradation during the sample treatment, making its recovery poor with common extraction methods. Other authors also reported recoveries around 60-70% for this compound in the LC-UV determination of linuron and three metabolites (3,4-dichloroaniline included) in potatoes [53]. A few compounds (carbendazim in three samples, and omethoate/ dimethoate in maracuya) could not be validated due to the presence of the analyte in the “blank” sample used in method validation. Recovery data obtained are shown in **Table 2, S I**.

Other parameters included in method validation were linearity of the calibration curve for standards in solvent, precision (expressed as repeatability, from recovery experiments) and limits of detection. The linearity was tested between 2.5–500 ng/mL (equivalent to 0.0025–0.5 mg/kg in sample). It was satisfactory in the majority of cases (commonly up to 250 ng/mL), with correlation coefficients above 0.99 and residuals lower than ± 20%. The LOQ objective was established as the lowest concentration that was validated in a fortified sample after application of the overall analytical procedure. According to our data, the LOQ objective was 0.01 mg/kg for the wide majority of compounds (**see Figure 4, and Table 2 SI**), as satisfactory recoveries (70-120%) and precision (RSD < 20%) were obtained at this level. No chromatographic peaks were observed in the processed blank samples; therefore, LODs as low as 0.5-3.0 µg/kg (0.0005 and 0.003 mg/kg) were estimated for a S/N=3 depending on the analyte/matrix combination.

In addition to the above indicated validation made for six selected matrices, the approach suggested in this work for matrix effects correction was supported by analysis of Quality Control (QC) samples that were included in the analysis sequence. QCs consisted on the same samples analyzed (12 samples from Spanish market and 12 samples collected directly from domestic Colombian markets) but previously fortified at 0.01 and 0.1 mg/kg. Thus, QC recoveries were obtained for all sample matrices, included those that were not subjected to validation (i.e. papaya, guayaba, feijoa, mangostan, lulo and carambolo).In this way every sample was analyzed as a “blank” (without fortification) and after fortification at two concentration levels as QCs (see next section), as explained in the next section.

3.5. Analysis of samples from the Spanish and Colombian markets

24 samples were analyzed following the procedure developed in this work. Two samples were analyzed for each type of matrix: one collected in Spain (although imported from Colombia) and the other collected directly in Bogotá domestic markets. QCs recovery data at 0.01 and 0.1 mg/kg allowed us to know whether the analytical methodology applied was adequate and whether matrix effects correction, using the correction factors previously calculated, was satisfactory. This was especially important for the six tropical matrices that had not been previously validated, and whose overall recoveries had not been calculated.

Data for QCs are shown in **Table 2**. As being an individual value, the acceptance criterion was 60-140%, in the line of the SANCO guideline for routine multi-residue analysis [39]. Among all QCs analysis, three individual recovery data were not

available due to the presence of the analyte in the sample at relatively high concentrations (these cases corresponded to carbendazim in papaya and lulo). So, from 228 possible recovery data (corresponding to 19 compounds x 12 matrices x 2 levels), up to 225 QCs recoveries were available. As it can be seen in **Table 2**, 202 out of 225 data were within the acceptable range. This corresponded to 90% of QC recoveries obtained. As expected from method validation for 3,4-dichloroaniline, several QCs recoveries for this compound were out of tolerance (mangostan, lulo and carambolo were around 40%). Apart from this analyte, the exceptions were mostly observed for methomyl and chlorpyrifos (5 data out of range), paraoxon methyl (4 data) and thiabendazole (2 data). Data for QCs in analyses of real-world samples, together with those obtained in method validation, support the applicability of the approach proposed in this work for matrix effects correction.

In relation to the positives found in samples, **Table 3** shows a summary of data obtained. 18 detections were found in the 12 samples from Spanish markets (emphasizing lulo sample with 5 positives and maracuyá with 3), while 16 detections were observed in the 12 samples from Colombian domestic market (emphasizing gulupa and carambolo with 3 positives each). In total, 9 different compounds were detected, and corresponded to 4 insecticides (methomyl, dimethoate, thiacloprid, carbofuran), 1 fungicide (carbendazim), 1 herbicide (diuron) and 3 metabolites (omethoate, clothianidin, paraoxon methyl). As stated before, the LOQ objective was 0.01 mg/kg as the method was not validated at concentrations below this value. However, the sensitivity was sufficient to allow estimating concentrations in positive samples far below the LOQ objective. In those cases, we could estimate the concentration in sample as the signal obtained was above S/N ratio of 10, commonly used as statistical LOQ of analytical methods. These values are marked by an asterisk in Table 3.

The compound most frequently detected, and at higher concentrations, was carbendazim that reached levels up to 3.4 mg/kg in papaya and was above 0.5 mg/kg in lulo and granadilla. This fungicide was mostly present in samples collected in Spain, which might imply that this compound was used as post-harvest fungicide during storage and transport. Apart from carbendazim, the rest of compounds did not exceed 0.1 mg/kg in samples, with the only exception of dimethoate in a maracuyá sample.

In **Figure 5**, several chromatograms for positive samples are shown as illustrative examples. In all cases, two transitions were acquired and the q/Q ion ratio was within the tolerances admitted (±30%) supporting the reliable identification of the compound detected in the sample.

The strategy proposed in this work is of easy application to other laboratories, which should estimate their own correction factors after performing an evaluation of matrix effects under their experimental conditions (as shown in sections 2.5 and discussed in section 3.3). After around 5 months that passed from matrix effects evaluation (estimation of correction factors) and analysis of the samples collected at Colombia, the correction factors were successfully applied to the QCs analyzed, showing the robustness of this approach in our laboratory. Correction factors would need to periodically be checked for possible changes in the MS and chromatographic conditions, and as also for different varieties of each food product to ensure an appropriate correction.

4. CONCLUSIONS

Many multiresidue pesticide methods have been reported in the scientific literature for fruits and vegetables. However, few methods have been specifically addressed to tropical food, which may become a problem when assessing the compliance of Maximum Residue Levels in these products. In this work, twelve tropical fruits highly popular in Colombia, with increasing relevance in international trade markets (carambolo, feijoa, granadilla, guayaba, gulupa, lulo, mangostan, maracuya, papaya, pithaya, tamarillo, uchuva), have been selected for the LC-MS/MS determination of 20 pesticides and metabolites. After using a QuEChERS-based sample treatment with acetonitrile as extracting solvent, a detailed study was made on matrix effects associated to the LC-MS/MS analysis. A series of correction factors have been proposed for each analyte/matrix combination in order to facilitate the accurate quantification of the compounds using calibration standards in solvent. By application of appropriate correction factors there was no need for using either isotope-labeled internal standards or matrix-matched calibration in every sequence of sample analysis for matrix effects correction. The methodology developed has been validated at 0.01 and 0.1 mg/kg levels in six sample matrices, and the usefulness of correction factors was tested in the rest of matrices by evaluating recoveries of quality control samples included in every sequence of sample analysis. Analysis of samples collected in Spain (exported from Colombia) and directly in Bogota domestic markets revealed the presence of some of the compounds under study (mainly the fungicide carbendazim, the insecticide dimethoate and its metabolite omethoate, and the insecticide thiacloprid). With the exception of carbendazim (the maximum level found was 3.4 mg/kg in a papaya sample), the rest of positives were below 0.2 mg/kg, the majority of

them being far below this value. MRLs set-up by the EU for these compounds [2] were exceeded for carbendazim in four samples (papaya, lulo, granadilla and maracuya, whose MRLs are between 0.1 mg/kg and 0.3 mg/kg), for dimetoathe in one maracuya sample (MRL 0.02 mg/kg), and diuron in one uchuva sample (MRL 0.01 mg/kg). It is worth noting that MRLs for these tropical fruits are commonly set at the default value corresponding to the limit of determination due to the lack of GLP studies on residue trials for these matrices. This fact makes that even small concentrations of pesticides in the samples may easily exceed the MRLs.

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Figure captions

Figure 1. Tropical Fruits studied in this work and MRLs groups as classified in Annex I "Products of plant and animal origin" [2]

Figure 2. Calibration graphs obtained for selected pesticides in different sample matrices. Calibration in solvent and ± 20% tolerance in the slope is highlighted in yellow

Figure 3. Difference (%) between matrix calibration and direct calibration slopes. Correction factors (in brackets) were applied only when difference was higher than ±20% (green/dark boxes and yellow/light boxes).

Figure 4. Average recoveries for selected pesticides in different sample matrices after application of the overall analytical procedure. Recovery data correspond to samples spiked at 0.01 and 0.1 mg/kg, and were calculated using calibration with standards in solvent with and without application of correction factors (see Figure 3 for correction factors)

Figure 5. Illustrative chromatograms for pesticides detected in tropical fruit samples. Q quantification transition. q confirmation transition. q/Q ratios in samples were within the maximum tolerances admitted in relation with those of reference standards

Table 1. MS/MS optimized conditions (ESI+) for the compounds and metabolites studied in this work.

Compound	tR (min)	Precursor ion (m/z)	Cone (V)	Collision Energy (eV)	Product ion (m/z)	q/Q Ratio
Omethoate (OME)	5.70	214.3	25	10 15	183.1 155	0.92
Carbendazim (CAR)	6.75	192.1	30	15 30	160.1 132.0	0.17
Methomyl (MTL)	6.98	163.1	20	10 10	88.0 106.0	0.77
Thiametoxam (THI)	7.26	292.0	25	15 25	211.2 181.2	0.66
Thiabendazole (THB)	7.56	202.3	35	25 30	175.1 131.2	0.63
Picloram (PIC)	7.87	241.1	25	20 30	195.2 168.0	0.58
Clothianidin (CLOT)	8.21	250.2	30	15 15	169.2 132.0	0.65
3-Hydroxycarbofuran (3-OH)	8.38	238.3	30	10 15	181.2 163.1	0.80
Dimethoate (DIM)	8.48	230.1	25	10 20	199.1 125.0	0.77
Thiacloprid (THC)	9.07	253.2	35	20 40	126.0 90.0	0.21
Paraoxon Methyl (PXON)	9.59	248.2	40	20 35	202.2 127.0	0.10
Imazalil (IMA)	9.63	297.2 299.2	35	25 20	159.1 161.1	0.73
Carbofuran (CRB)	9.98	222.2	30	10 20	165.1 123.1	0.79

Malaoxon (MLX)	10.08	315.2	30	15 10	127.1 143.0	0.15
3,4-dichlooraniline (3,4 DCA)	10.56	162.1 164.1	30	20 20	127.0 129.0	0.33
Diuron (DIU)	11.06	233.1 235.2	35	15 10	71.9 71.9	0.31
Clomazone (CLO)	11.19	240.2 242.2	30	20 20	125.1 127.1	0.32
Parathion- methyl (PAR)	11.44	264.2	40	15 20	125.1 143.2	0.10
Malathion (MAL)	11.69	331.1	30	15 10	127.0 285.0	0.43
Chlorpyrifos (CHLOR)	13.37	350.0 352.0	30	20 20	198.1 200.1	0.97

Table 2. Recoveries (%) obtained for quality controls (at 0.01 and 0.1 mg/kg level) that were analyzed in the sample sequence for six tropical fruit matrices. Concentrations calculated using calibration standards in solvent and applying the corresponding correction factors (see Figure 3)

Matrix	Papaya		Guayaba		Feijoa		Mangostan		Lulo		Carambolo	
	0.01	*0.1*	*0.01*	*0.1*	*0.01*	*0.1*	*0.01*	*0.1*	*0.01*	*0.1*	*0.01*	*0.1*
Pesticide												
Omethoate	80	78	74	83	91	75	81	62	64	96	89	92
Methomyl	88	61	64	46	84	66	79	46	43	40	74	46
Thiametoxan	121	101	96	74	112	99	78	60	60	70	130	100
Carbendazim	*	*	68	64	111	94	70	63	*	91	85	92
Clothianidin	128	110	80	70	107	98	66	67	69	73	116	87
3OH-Carbofuran	78	74	84	72	120	112	85	74	62	68	110	86
Dimethoate	97	93	101	89	107	101	92	80	66	78	123	105
Thiabendazole	74	51	70	60	75	66	62	50	133	78	76	60
Thiacloprid	98	92	104	85	112	98	83	84	61	67	104	79
Paraoxon methyl	153	155	120	111	150	150	95	82	89	93	140	123
Carbofuran	85	103	69	77	104	120	95	110	52	85	83	83
Imazalil	63	63	66	61	111	91	117	60	62	61	69	64
Malaoxon	111	105	71	67	107	99	85	92	70	103	77	74
3,4 Dicloroaniline	88	100	60	62	75	63	47	39	47	47	41	47
Diuron	83	84	110	96	109	100	106	77	86	90	128	109
Clomazone	89	90	98	86	116	110	112	89	78	87	111	92
Parathion Methyl	97	85	100	81	131	89	77	83	76	85	116	72
Malathion	71	86	77	82	128	140	140	131	84	118	89	89
Chlorpyrifos	175	179	113	99	172	162	71	61	125	127	182	124

*Not determined because of the presence of analyte in unfortified sample

Table 3. Pesticide concentrations (µg/kg) in the tropical fruits analyzed: (1) samples used for validation, collected from Spanish markets, (2) samples collected from Colombian domestic markets

Sample / Pesticide	Lulo	Carambolo	Granadilla	Mangostan	Tamarillo	Gulupa	Maracuya	Uchuva	Guayaba	Pithaya	Papaya	Feijoa
Omethoate	2.9(1)*	-	-	-	2.1(1)*	d(2)	42(1)	-	-	-	-	-
Methomyl	1.5(1)*	-	-	-	-	-	-	-	-	-	-	-
Carbendazim	1340(1) 19(2)	2.1(1)* d (2)	660(1) -	3.5(1)* -	290(1) 80(2)	7.2(1)* 2.6(2)*	210(1) 30(2)	-	d(1) d(2)	- d(2)	3400(1) -	d(1) 3.2(2)*
Clothianidin	-	-	-	-	2.2(2)*	-	-	-	-	-	-	-
Dimethoate	1.6(1)*	10(2)	-	-	-	2.0(2)*	160(1)	-	-	-	-	-
Thiacloprid	8.0(1)*	d (2)	-	-	-	-	-	-	-	-	-	-
Carbofuran	d(2)	-	-	-	-	-	-	-	-	-	-	d (2)
Diuron	-	-	-	-	-	-	-	50(2)	-	-	-	-
Paraoxon Methyl	-	-	-	-	-	-	-	-	-	-	-	14(1)

d: *detected*

**: estimated concentration corresponding to a response above S/N ratio of 10 (below the LOQ objective of 0.01 mg/kg).*

Fruit	Scientific name	Family	MRL Group, Annex I
 Mangostan	*Garcinia mangostana*	Clusiaceae	**1.Fruits fresh** (vi): Miscellaneous fruit (b): Inedible peel,small -Lychee
 Pithaya (1)	*Hylocereus Selenicereus*	Cactaceae	**1.Fruits fresh** (vi): Miscellaneous fruit (c): Inedible peel, large -(1) Guava -(2) Papaya
 Guayaba (1)	*Psidium guajava*	Myrtaceae	
 Feijoa (1)	*Feijoa sellowiana*	Myrtaceae	
 Papaya (2)	*Carica papaya*	Caricaceae	
 Carambolo	*Averrhoa carambola*	Oxalidaceae	**1.Fruits fresh** (vi): Miscellaneous fruit (a): Edible peel -Carambolo

Fruit	Scientific name	Family	MRL Group, Annex I
 Maracuya	*Passiflora edulis*	Passifloraceae	**1.Fruits fresh** (vi): Miscellaneous fruit (b): Inedible peel,small - Passion Fruit
 Gulupa	*Passiflora pinnatistipula*	Passifloraceae	
 Granadilla	*Passiflora ligularis*	Passifloraceae	
 Tamarillo (3)	*Cyphomandra betacea*	Solanaceae	**2.Vegetables fresh** (iii):Fruitingvegatables (a): Solanacea -(3) Tomatoes -(4) Others
 Uchuva (3)	*Physalis peruvianaL.*	Solanaceae	
 Lulo (4)	*Solanum quitoense*	Solanaceae	

Figure 1

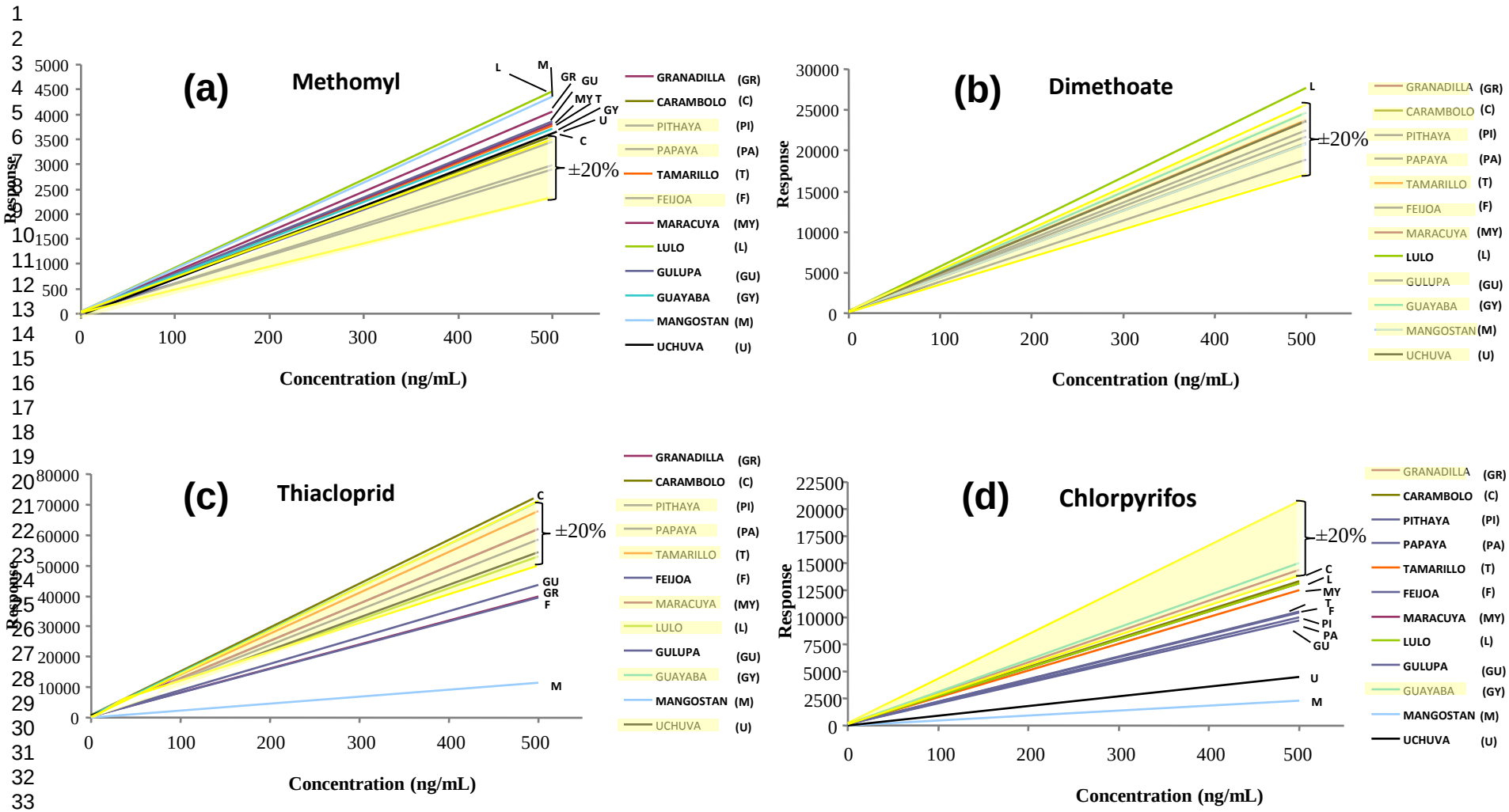
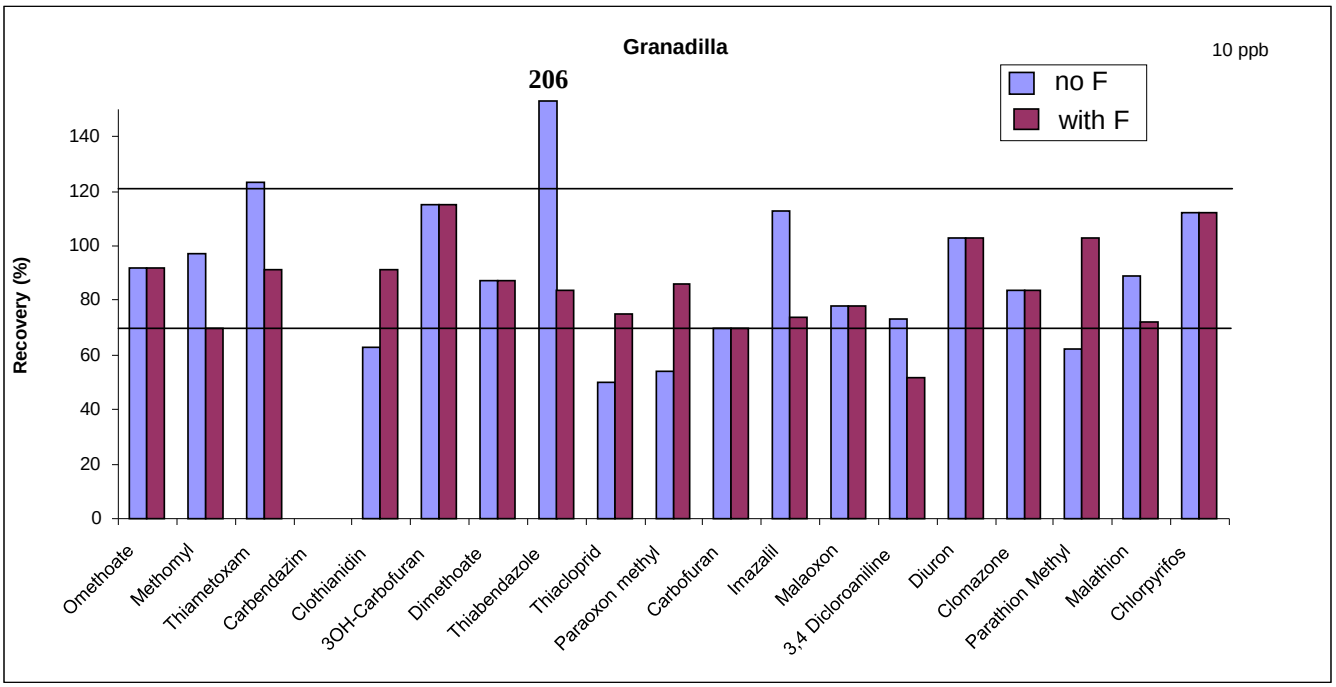

(a)
Methomyl
Response
Concentration (ng/mL)
±20%
L
M
GR
GU
MY
T
GY
U
C
(b)
Dimethoate
(c)
Thiacloprid
(d)
Chlorpyrifos
GRANADILLA (GR)
CARAMBOLO (C)
PITHAYA (PI)
PAPAYA (PA)
TAMARILLO (T)
FEIJOA (F)
MARACUYA (MY)
LULO (L)
GULUPA (GU)
GUAYABA (GY)
MANGOSTAN (M)
UCHUVA (U)

Figure 2

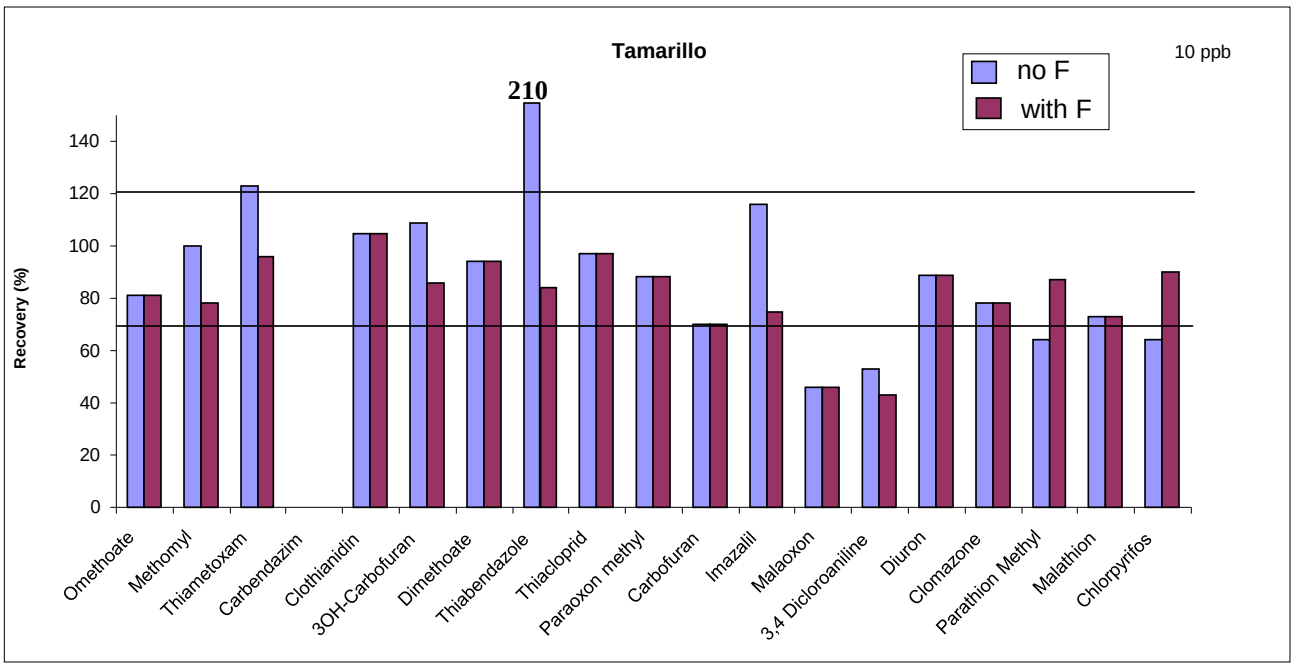
Compound	Granadilla	Lulo	Guayaba	Mangostan	Uchuva	Maracuya	Pithaya	Carambolo	Gulupa	Papaya	Tamarillo	Feijoa
Omethoate	11 (0.90)	−11 (1.12)	−10 (1.11)	−7 (1.08)	−2 (1.02)	20 (0.83)	−6 (1.06)	−3 (1.03)	13 (0.88)	−11 (1.12)	17 (0.85)	−8 (1.09)
Methomyl	39 (0.72)	53 (0.65)	27 (0.79)	49 (0.67)	26 (0.79)	31 (1.45)	18 (0.85)	22 (0.82)	32 (0.76)	2 (0.98)	29 (0.78)	−1 (1.01)
Thiametoxam	35 (0.74)	30 (0.77)	32 (0.76)	22 (0.82)	54 (0.65)	38 (0.72)	42 (0.70)	22 (0.82)	−3 (1.03)	7 (0.93)	28 (0.78)	9 (0.92)
Carbendazim	− 18 (1.22)	−55 (2.22)	26 (0.79)	24 (0.81)	−15 (1.18)	−22 (1.28)	18 (1.22)	−8 (1.09)	−16 (1.19)	−58 (2.38)	10 (0.91)	−12 (1.14)
Clothianidin	−31 (1.45)	−2 (0.79)	23 (0.81)	−25 (1.33)	11 (0.90)	13 (0.88)	−15 (1.18)	36 (0.74)	−1 (1.01)	11 (0.90)	15 (0.87)	−7 (1.08)
3 OH Carbofuran	18 (0.85)	53 (0.65)	29 (0.78)	25 (0.80)	32 (0.76)	21 (0.83)	18 (0.85)	24 (0.81)	14 (0.88)	22 (0.82)	26 (0.79)	4 (0.96)
Dimethoate	−3 (1.03)	29 (0.78)	15 (0.87)	−3 (1.03)	10 (0.91)	10 (0.91)	5 (0.95)	10 (0.91)	−2 (1.02)	1 (0.99)	11 (0.90)	−12 (1.14)
Thiabendazol	145 (0.41)	94 (0.52)	153 (0.40)	103 (0.49)	145 (0.41)	53 (0.65)	178 (0.36)	221 (0.31)	37 (0.73)	169(0.37)	155 (0.40)	153 (0.40)
Thiacloprid	−33 (1.49)	−11(1.12)	19 (0.84)	−81 (5.26)	−9 (1.10)	4 (0.96)	−2 (1.02)	21 (0.83)	− 27(1.37)	4 (0.96)	14 (0.88)	−34 (1.52)
Paraoxon methyl	−37 (1.59)	−10 (1.11)	7 (0.93)	−74 (3.85)	−20 (1.25)	−11 (1.12)	−25 (1.33)	0 (1.0)	−53 (2.13)	−34 (1.52)	−8 (1.09)	−43 (1.75)
Carbofuran	−17 (1.20)	−10 (1.11)	−14 (1.22)	−51 (2.04)	−30 (1.43)	−12 (1.14)	−11 (1.12)	0 (1.0)	−17 (1.20)	−26 (1.35)	−18 (1.22)	−26 (0.85)
Imazalil	36 (0.74)	119 (0.46)	94 (0.52)	21 (0.83)	54 (0.65)	46 (0.68)	45 (0.69)	103 (0.49)	23 (0.82)	89 (0.53)	53 (0.65)	34 (0.74)
Malaoxon	− 2 (1.02)	−21 (1.27)	−11 (1.12)	−33 (2.49)	−21 (1.27)	−24 (1.32)	1 (1.01)	−17 (1.20)	−15 (1.18)	−39 (1.64)	−20 (1.25)	−29 (1.41)
3.4 Dichloroaniline*	41 (0.71)	27(0.79)	27 (0.79)	12 (0.89)	0 (1.0)	26 (0.79)	29 (0.78)	38 (0.72)	11 (0.90)	16 (0.86)	23 (0.81)	14 (0.88)
Diuron	17 (0.85)	11 (0.90)	10 (0.90)	−50 (2.0)	−7 (1.08)	12 (0.89)	−12 (1.14)	16 (0.86)	−2 (1.02)	−16 (1.19)	9 (0.92)	−13 (1.15)
Clomazone	6 (0.94)	9 (0.92)	10 (0.91)	−57(2.33)	− 50 (2.0)	4 (0.96)	−12 (1.14)	9 (0.92)	−7 (1.08)	3 (0.97)	5 (0.95)	−22 (1.28)
Parathion methyl	−40 (1.69)	−23 (1.30)	−30 (1.43)	−66 (2.94)	−34 (1.52)	−21 (1.27)	−25 (1.33)	−16 (1.19)	−47 (1.89)	−38 (1.61)	−26 (1.35)	−44 (1.79)
Malathion	23 (0.81)	6 (0.94)	45 (0.69)	−53 (2.13)	15 (0.87)	28 (0.78)	10 (0.91)	28 (0.78)	22 (0.82)	23 (0.81)	18 (0.85)	9 (0.92)
Chlorpyrifos	−17 (1.20)	−24 (1.16)	−13 (1.15)	−87(7.69)	−74 (3.85)	−24 (1.32)	−40(1.67)	−23 (1.30)	−44 (1.30)	− 42 (1.72)	−28 (1.39)	−39 (1.64)

± 0-20% > 20% < 20%

Figure 3

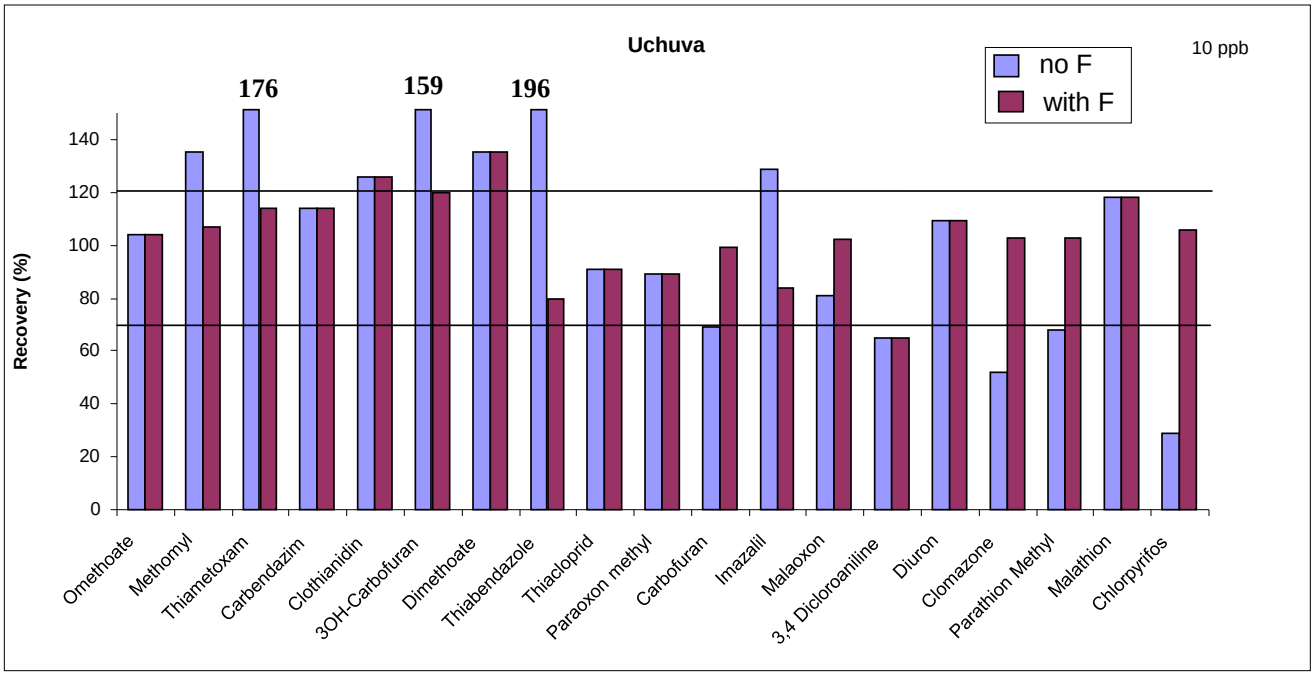

Granadilla
10 ppb
206
no F
with F
Recovery (%)
0
20
40
60
80
100
120
140
Omethoate
Methomyl
Thiametoxam
Carbendazim
Clothianidin
3OH-Carbofuran
Dimethoate
Thiabendazole
Thiacloprid
Paraoxon methyl
Carbofuran
Imazalil
Malaoxon
3,4 Dicloroaniline
Diuron
Clomazone
Parathion Methyl
Malathion
Chlorpyrifos

(a)

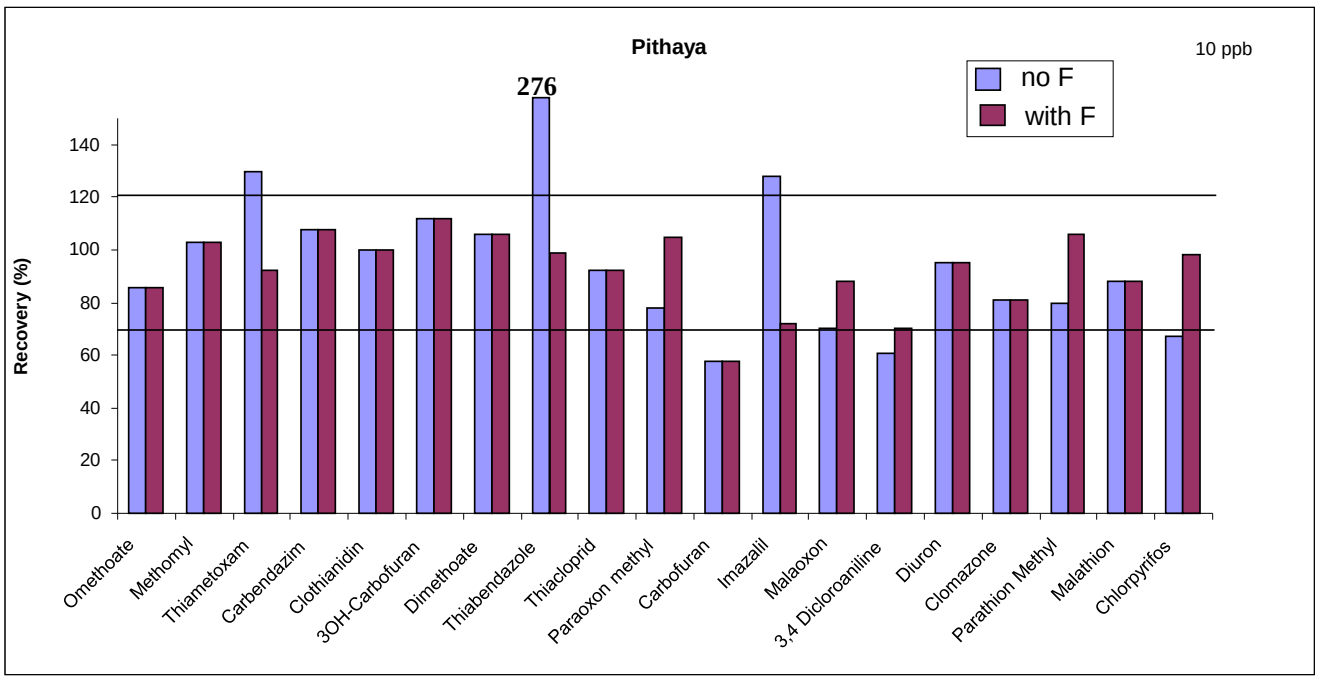

Tamarillo
10 ppb
210
no F
with F
Recovery (%)
0
20
40
60
80
100
120
140
Omethoate
Methomyl
Thiametoxam
Carbendazim
Clothianidin
3OH-Carbofuran
Dimethoate
Thiabendazole
Thiacloprid
Paraoxon methyl
Carbofuran
Imazalil
Malaoxon
3,4 Dicloroaniline
Diuron
Clomazone
Parathion Methyl
Malathion
Chlorpyrifos

(b)

Figure 4

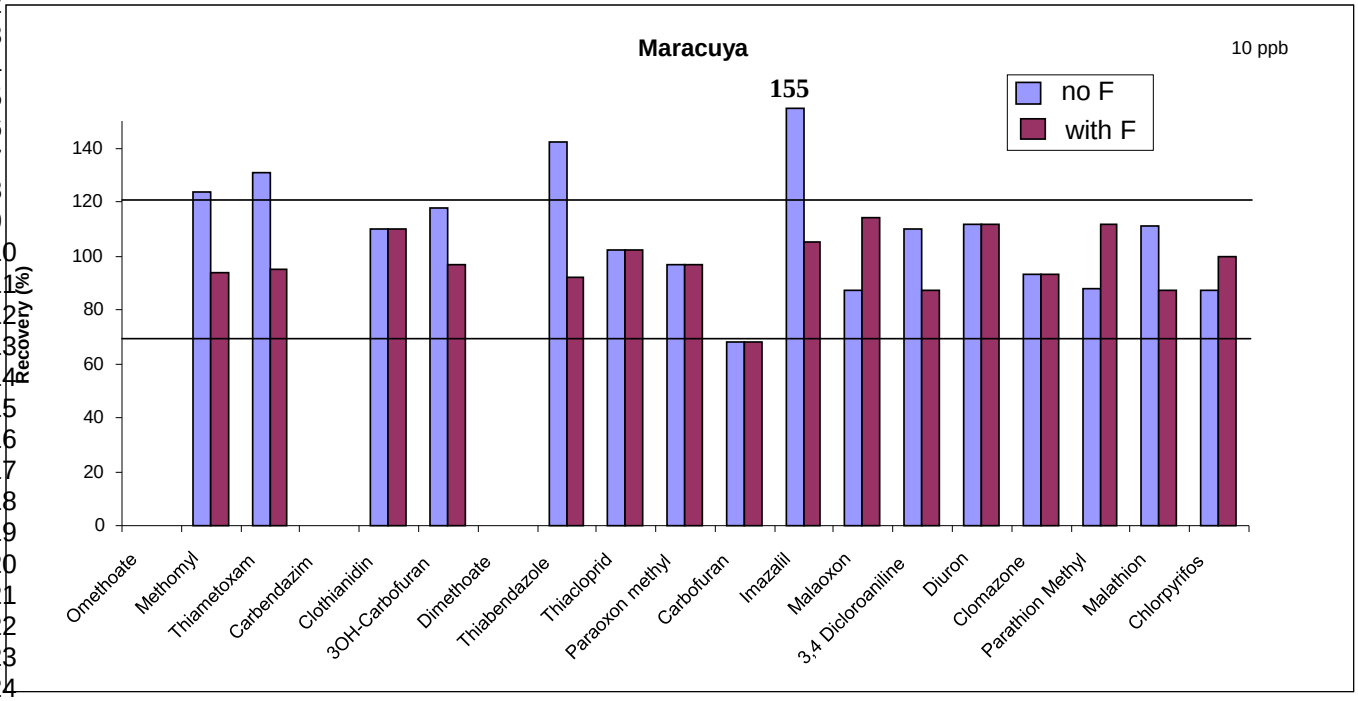


(c)

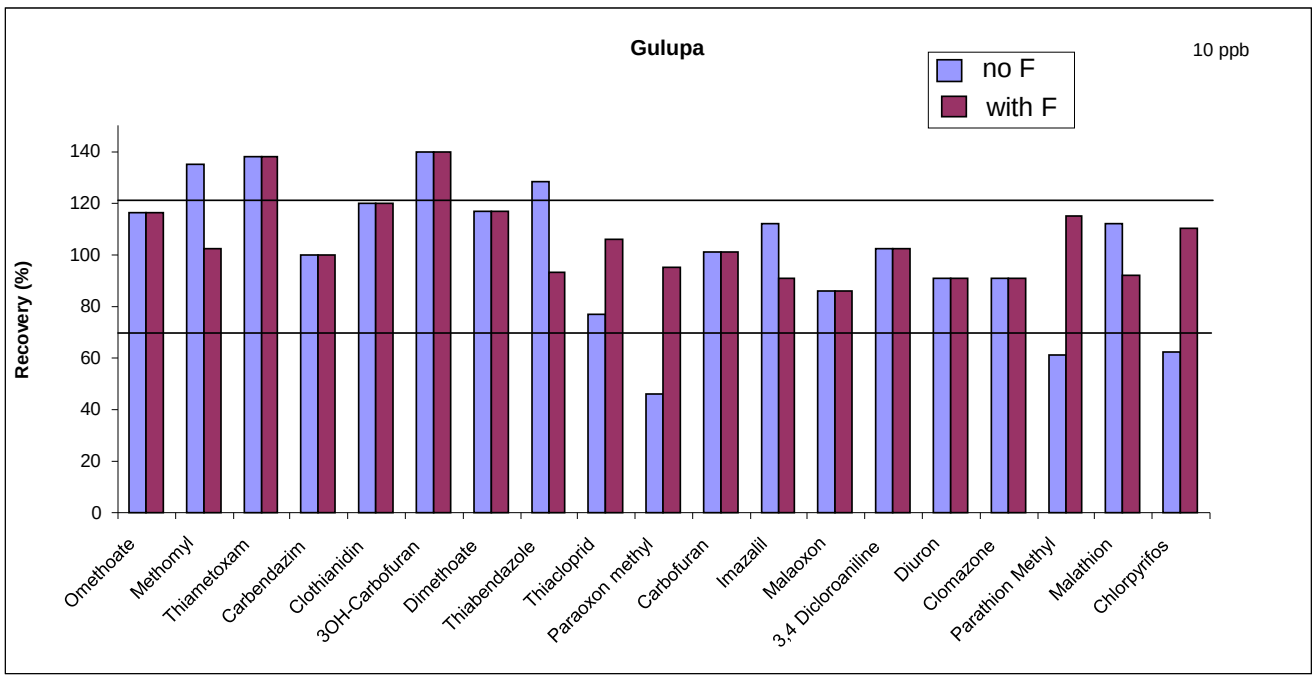


(d)

Figure 4 (cont)



(e)



(f)

Figure 4 (cont)

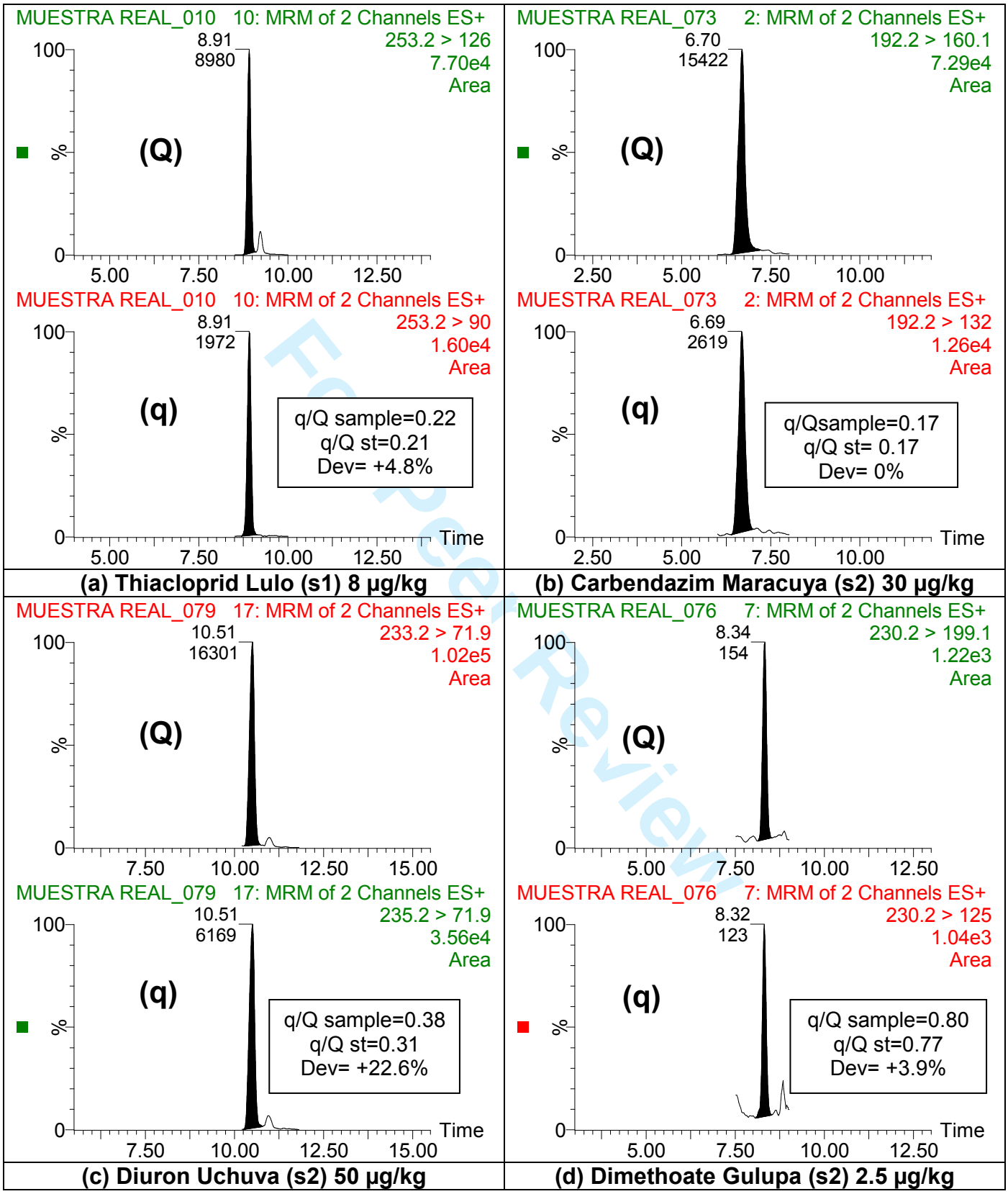


Figura 5

SUPLEMENTARY INFORMATION

Exploring matrix effects in liquid chromatography-tandem mass spectrometry determination of pesticide residues in tropical fruits

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In this section, three figures and two tables are included giving useful supplementary information for the readers.

Table SI 1. Compounds and metabolites included in this work. Maximum Residue Limits (MRLs) as established in Regulation (EC) No 396/2005

Pesticide	Use	Metabolite	Granadilla	Maracuya	Gulupa	Mangostan	Tamarillo	Uchuva	Lulo	Carambolo	Feijoa	Guayaba	Pithaya	Papaya
Methomyl	insecticide		0,02*	0,02*	0,02*	0,02*	0,02*	0,02*	0,02*	0,05*	0,05*	0,05*	0,05*	0,02*
Thiamethoxam	insecticide	clothianidin	0,05*	0,05*	0,05*	0,05*	**0,2**	**0,2**	0,05*	0,05*	0,05*	0,05*	0,05*	0,05*
Carbendazim	fungicide		0,1*	0,1*	0,1*	0,1*	**0,3**	**0,3**	0,1*	0,1*	0,1*	0,1*	0,1*	**0,2**
Dimethoate	insecticide	omethoate	0,02*	0,02*	0,02*	0,02*	0,02*	0,02*	0,02*	0,02*	0,02*	0,02*	0,02*	0,02*
Thiacloprid	insecticide		0,02*	0,02*	0,02*	0,02*	0,5	0,5	0,02*	0,02*	0,02*	0,02*	0,02*	**0,5**
Thiabendazole	fungicide		0,05*	0,05*	0,05*	0,05*	0,05*	0,05*	0,05*	0,05*	0,05*	0,05*	0,05*	**10**
Carbofuran	insecticide	3-OH-carbofuran	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*
Imazalil	fungicide		0,05*	0,05*	0,05*	0,05*	**0,5**	**0,5**	0,05*	0,05*	0,05*	0,05*	0,05*	0,05*
Picloram	herbicide		0,01*	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*
Diuron	herbicide	3,4- dichloraniline	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*
Clomazone	herbicide		0,01*	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*
Malathion	insecticide-acaricide	malaoxon	0,02*	0,02*	0,02*	0,02*	0,02*	0,02*	0,02*	0,02*	0,02*	0,02*	0,02*	0,02*
Parathion-methyl	insecticide, acaricide	paraoxon-methyl	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*	0,01*
Chlorpyrifos	insecticide		0,05*	0,05*	0,05*	0,05*	**0,5**	**0,5**	0,05*	0,05*	0,05*	0,05*	0,05*	0,05*

*Limit of determination of the analytical method

Bold number indicate that GLP residue trials have been performed in order to set up the MRL

Table SI 2a. Recoveries (%) obtained for the six matrices subjected to validation using concentrations calculated with direct calibration (*direct*) and corrected after application of the matrix effect factors (*corrected*). Fortification level 0.01 mg/kg (n=5)

Matrix	Granadilla		Tamarillo		Uchuva		Pithaya		Maracuya		Gulupa	
	direct	*corrected*	*direct*	*corrected*	*direct*	*corrected*	*direct*	*corrected*	*direct*	*corrected*	*direct*	*corrected*
Pesticide												
Omethoate	92	92	81	81	104	104	86	86	*	*	116	116
Methomyl	97	70	100	78	135	107	103	103	124	94	135	102
Thiametoxan	123	91	123	96	176	114	130	92	131	95	138	138
Carbendazim	*	*	*	*	114	114	108	108	*	*	100	100
Clothianidin	63	91	105	105	126	126	100	100	110	110	120	120
3OH-Carbofuran	115	115	109	86	159	120	112	112	118	97	140	140
Dimethoate	87	87	94	94	135	135	106	106	*	*	117	117
Thiabendazole	206	84	210	84	196	80	276	99	142	92	128	93
Thiacloprid	50	75	97	97	91	91	92	92	102	102	77	106
Paraoxon methyl	54	86	88	88	89	89	78	105	97	97	46	95
Carbofuran	70	70	70	70	69	99	58	58	68	68	101	101
Imazalil	113	74	116	75	129	84	128	88	155	105	112	91
Malaoxon	78	78	46	46	81	102	70	70	87	114	86	86
3,4 Dicloroaniline	73	52	53	43	65	65	61	47	110	87	102	102
Diuron	103	103	89	89	109	109	95	95	112	112	91	91
Clomazone	84	84	78	78	52	103	81	81	93	93	91	91
Parathion Methyl	62	103	64	87	68	103	80	106	88	112	61	115
Malathion	89	72	73	73	118	118	88	88	111	87	112	92
Chlorpyrifos	112	112	64	90	29	106	67	98	87	100	62	110

* Data not available due to the presence of the analyte in the sample used for validation

Table SI 2b. Recoveries (%) obtained for the six matrices subjected to validation using concentrations calculated with direct calibration (*direct*) and corrected after application of the matrix effect factors (*corrected*). Fortification level 0.1 mg/kg (n=5)

Matrix	Granadilla		Tamarillo		Uchuva		Pithaya		Maracuya		Gulupa	
	direct	*corrected*	*direct*	*corrected*	*direct*	*corrected*	*direct*	*corrected*	*direct*	*corrected*	*direct*	*corrected*
Pesticide												
Omethoate	103	103	84	84	108	108	85	85	84	84	117	117
Methomyl	106	76	103	80	136	109	104	104	127	97	129	98
Thiametoxan	137	101	108	84	161	104	122	86	136	99	104	104
Carbendazim	*	*	50	50	107	107	105	105	86	110	108	108
Clothianidin	70	102	96	96	127	127	94	94	118	97	114	114
3OH-Carbofuran	122	122	98	78	159	120	116	116	126	104	124	124
Dimethoate	92	92	88	88	131	131	104	104	97	97	110	110
Thiabendazole	204	83	184	74	187	76	242	87	125	81	57	71
Thiacloprid	53	79	88	88	87	87	92	92	95	95	69	95
Paraoxon methyl	55	88	77	77	84	84	76	102	93	93	44	94
Carbofuran	81	81	79	79	85	120	60	60	84	84	106	106
Imazalil	113	74	111	72	130	84	110	76	117	80	106	86
Malaoxon	86	86	55	55	98	124	77	77	96	127	92	92
3,4 Dicloroaniline	70	50	52	42	63	63	49	38	83	68	78	78
Diuron	104	104	84	84	107	107	92	92	110	110	87	87
Clomazone	88	88	77	77	52	104	81	81	95	95	87	87
Parathion Methyl	58	94	57	77	60	90	64	85	92	117	63	119
Malathion	103	83	79	79	127	127	96	96	118	92	115	94
Chlorpyrifos	73	73	59	81	28	102	55	92	76	100	64	114

* Data not available due to the presence of the analyte in the sample used for validation

Figure SI 1. QuEChERS procedure applied in this work

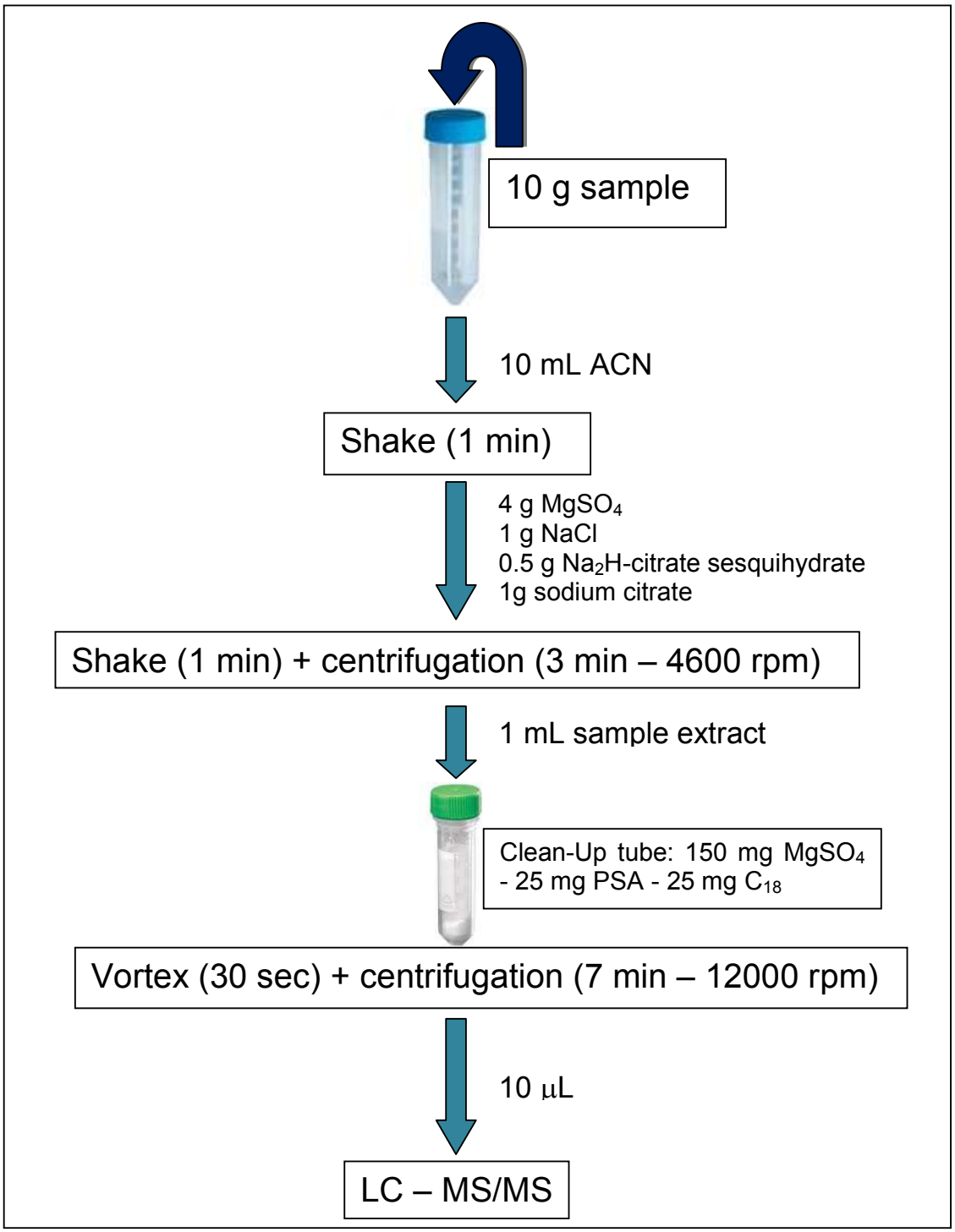


Figure SI 2. Correction factor calculation

$$\frac{Area}{Direct\ Slope} \times F = \frac{Area}{Direct\ Slope\ +\ ((\%\ Difference\ \times\ Direct\ Slope)/100)}$$

$$\frac{F}{Direct\ Slope} = \frac{1}{Direct\ Slope + ((\%\ Difference \times Direct\ Slope)/100)}$$

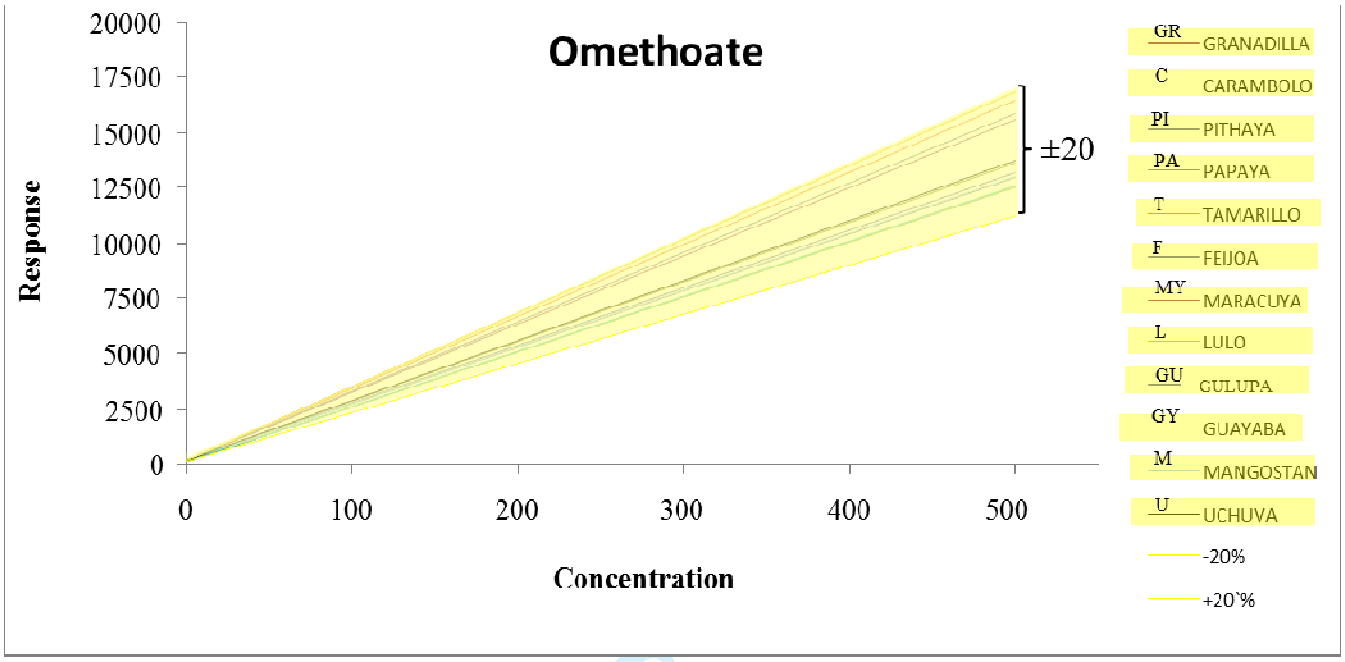
$$F = \frac{Direct\ slope}{Direct\ Slope\ +\ ((\%\ Difference\ \times Direct\ Slope)/100)}$$

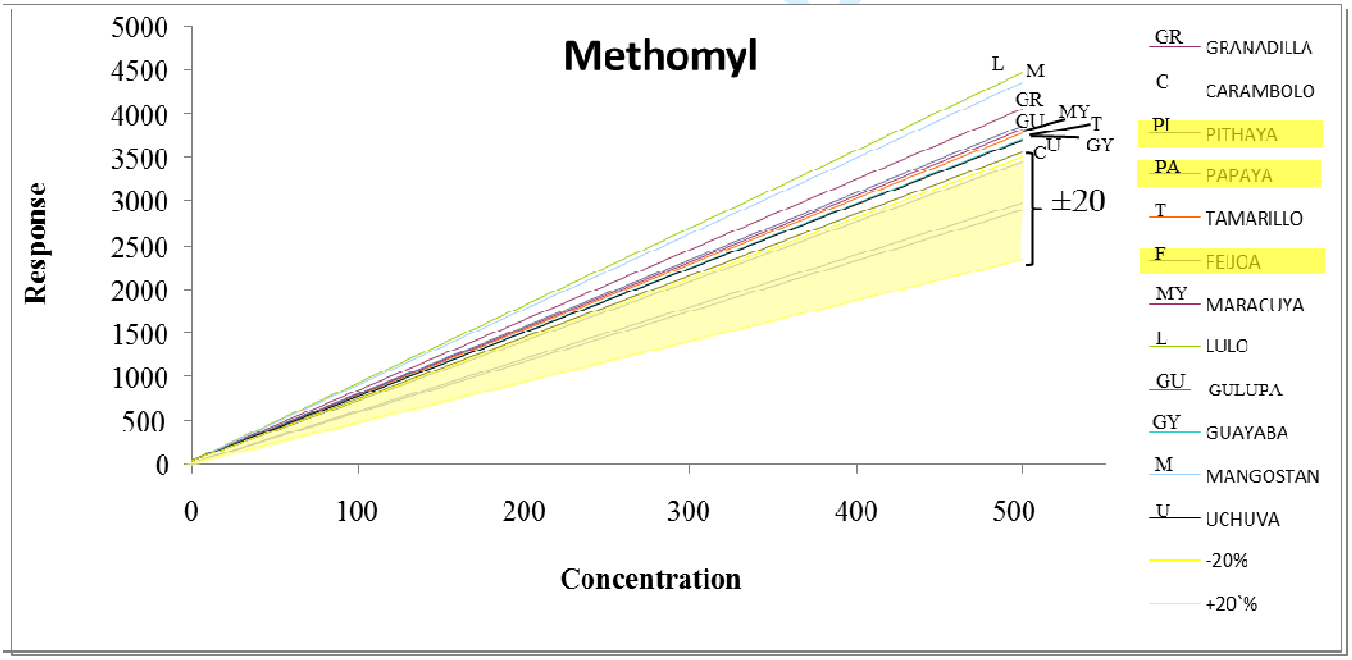
$$F = \frac{Direct\ Slope}{\frac{100\ \times Direct\ Slope}{100} + \frac{(\%\ Difference\ \times Direct\ Slope}{100}}$$

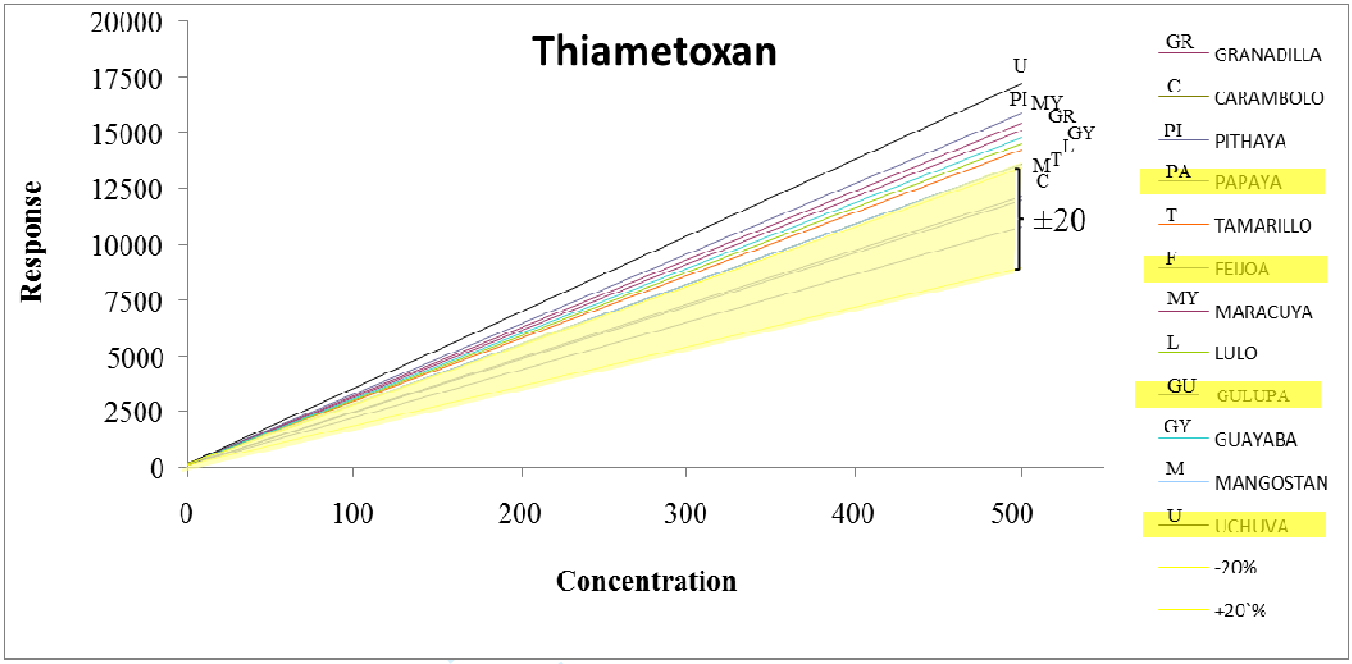
$$F = \frac{Direct\ Slope}{Direct\ Slope\ \times \left[1 + \frac{\%\ Difference}{100}\right]}$$

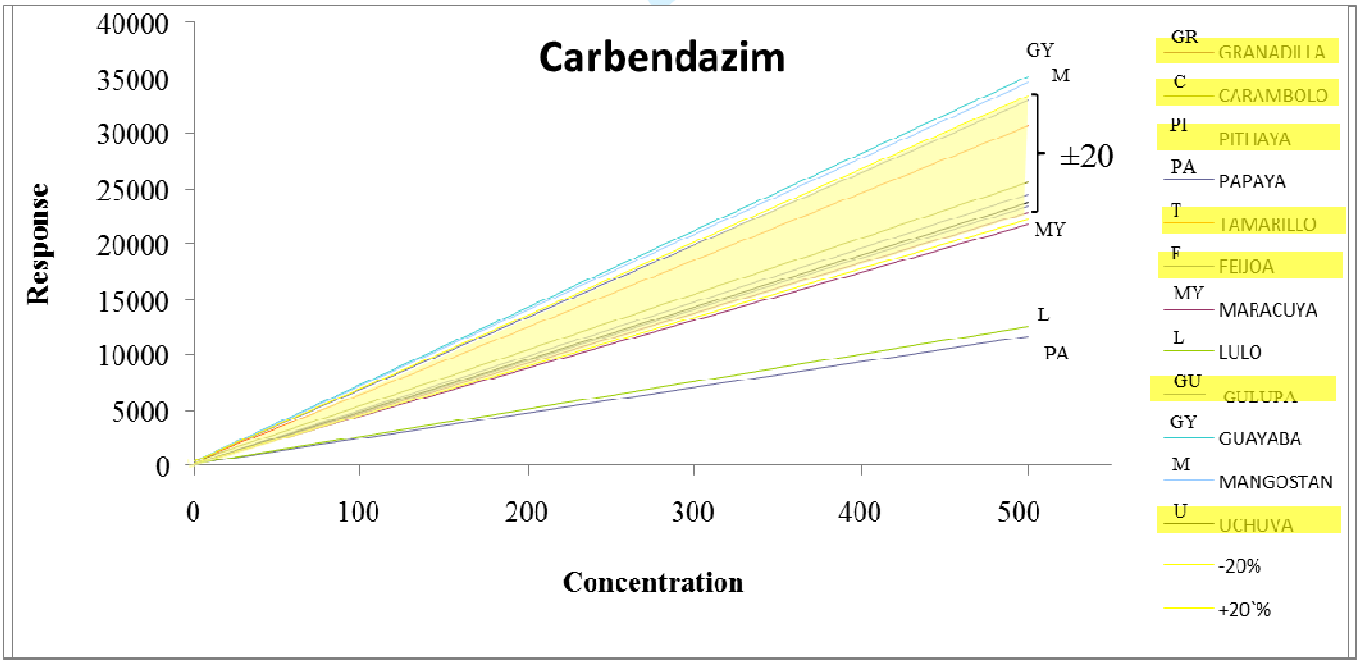
$$F = \frac{1}{1 + \frac{\%\ Difference}{100}}$$

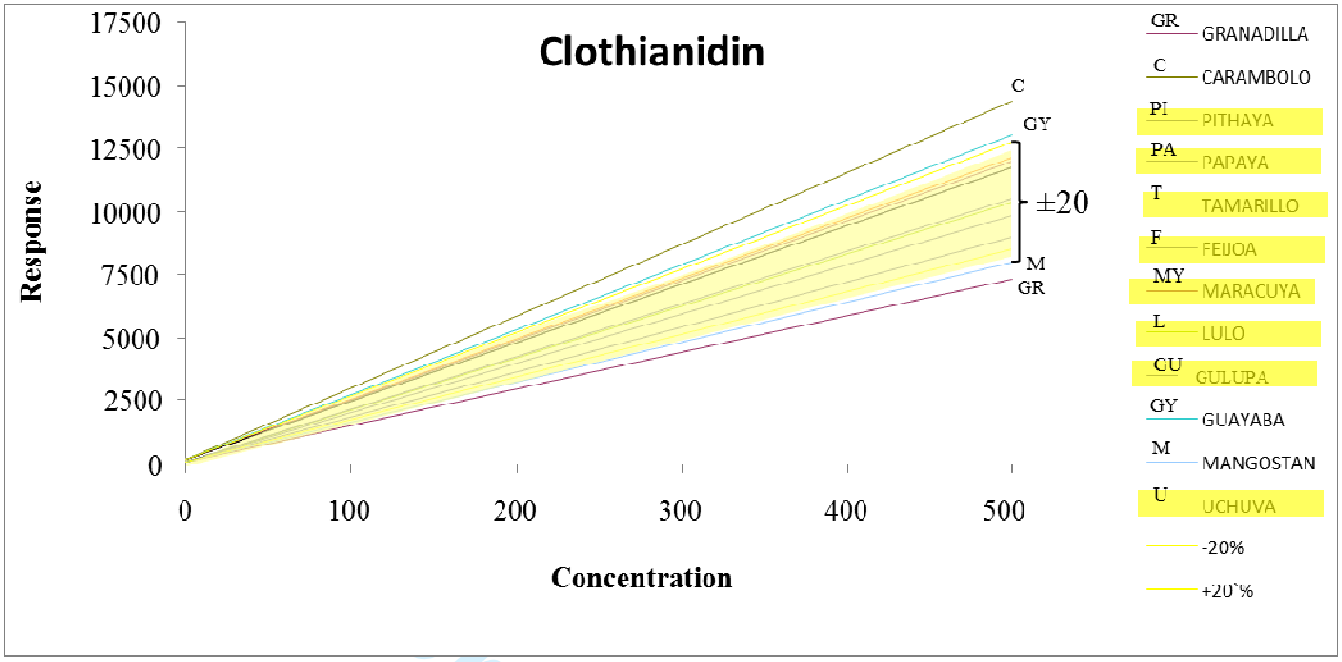
Figure SI 3. Calibration graphs obtained for selected pesticides in different sample matrices. Calibration in solvent and ± 20% tolerance in the slope is highlighted in yellow

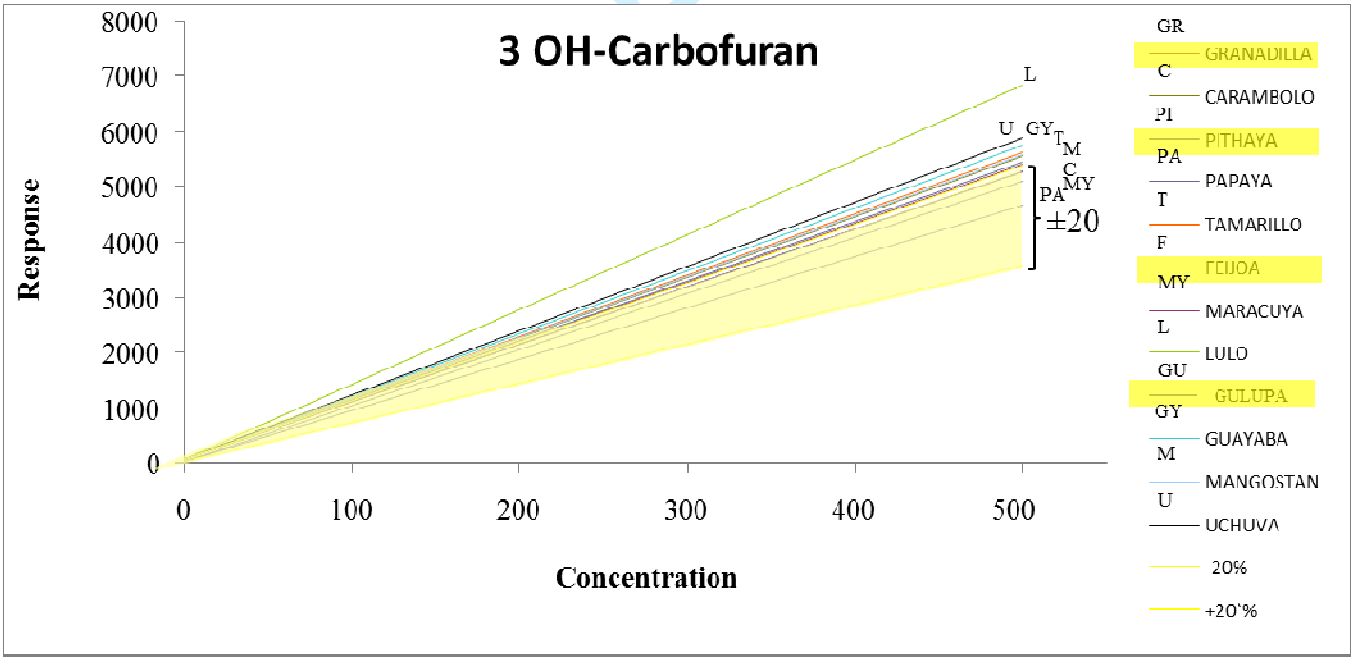


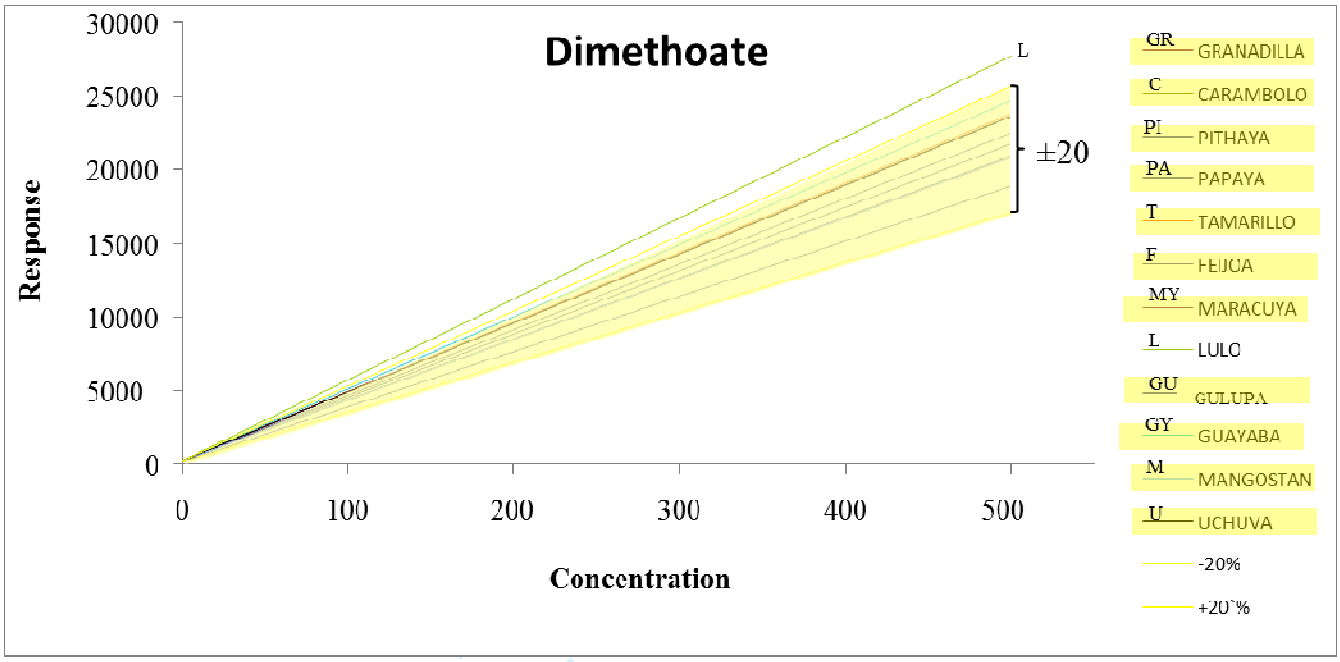


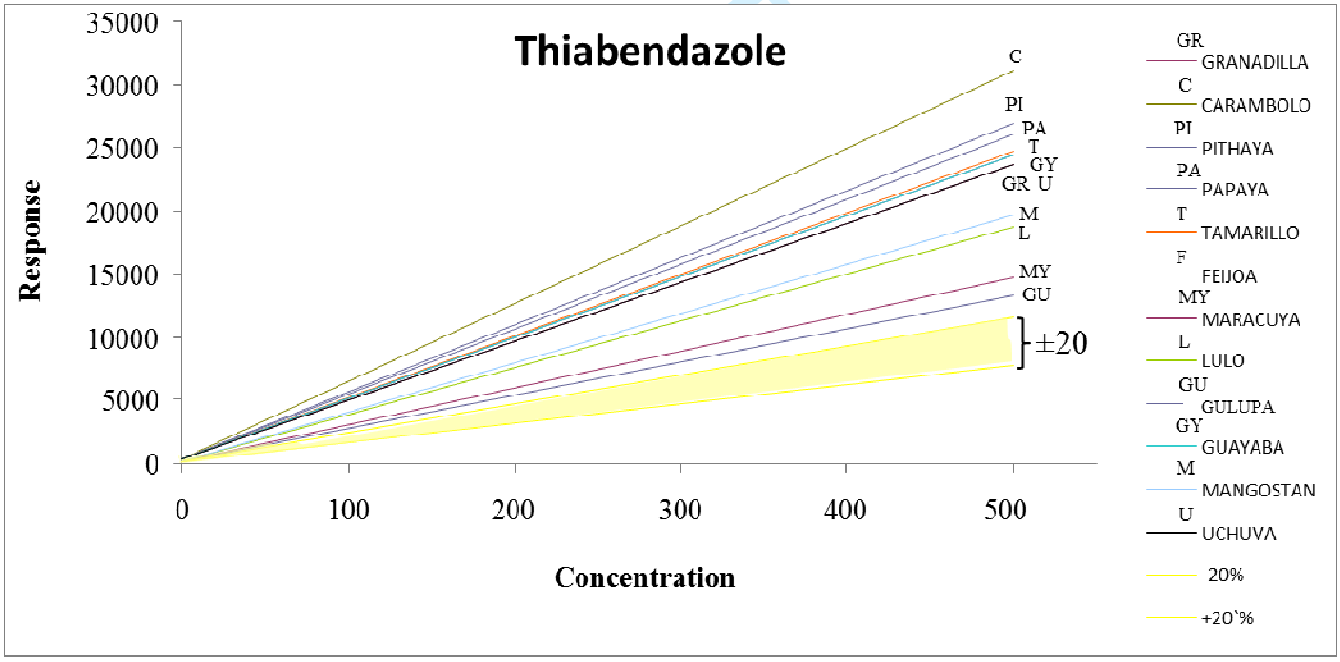

Thiametoxan
Response
Concentration
20000
17500
15000
12500
10000
7500
5000
2500
0
0
100
200
300
400
500
U
PI
MY
GR
GY
L
T
M
C
±20
GR GRANADILLA
C CARAMBOLO
PI PITHAYA
PA PAPAYA
T TAMARILLO
F FEIJOA
MY MARACUYA
L LULO
GU GULUPA
GY GUAYABA
M MANGOSTAN
U UCHUVA
-20%
+20`%

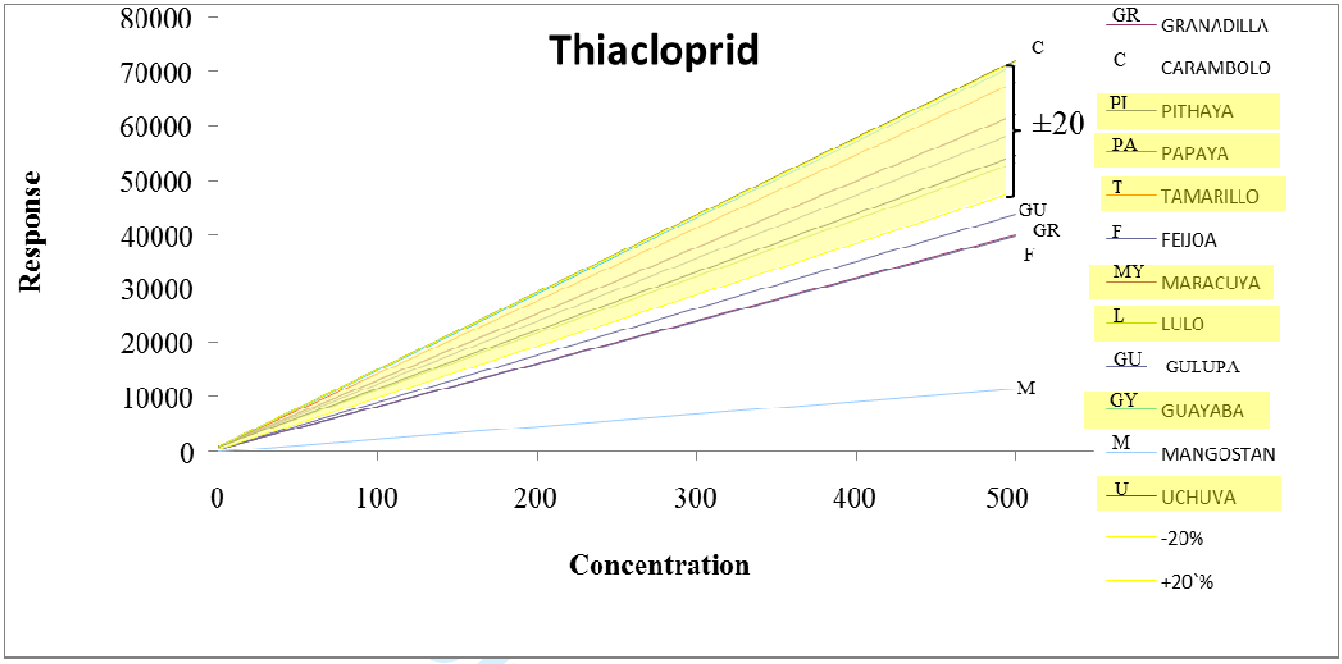

Carbendazim
Response
Concentration
40000
35000
30000
25000
20000
15000
10000
5000
0
0
100
200
300
400
500
GY
M
±20
MY
L
PA
GR GRANADILLA
C CARAMBOLO
PI PITHAYA
PA PAPAYA
T TAMARILLO
F FEIJOA
MY MARACUYA
L LULO
GU GULUPA
GY GUAYABA
M MANGOSTAN
U UCHUVA
-20%
+20`%

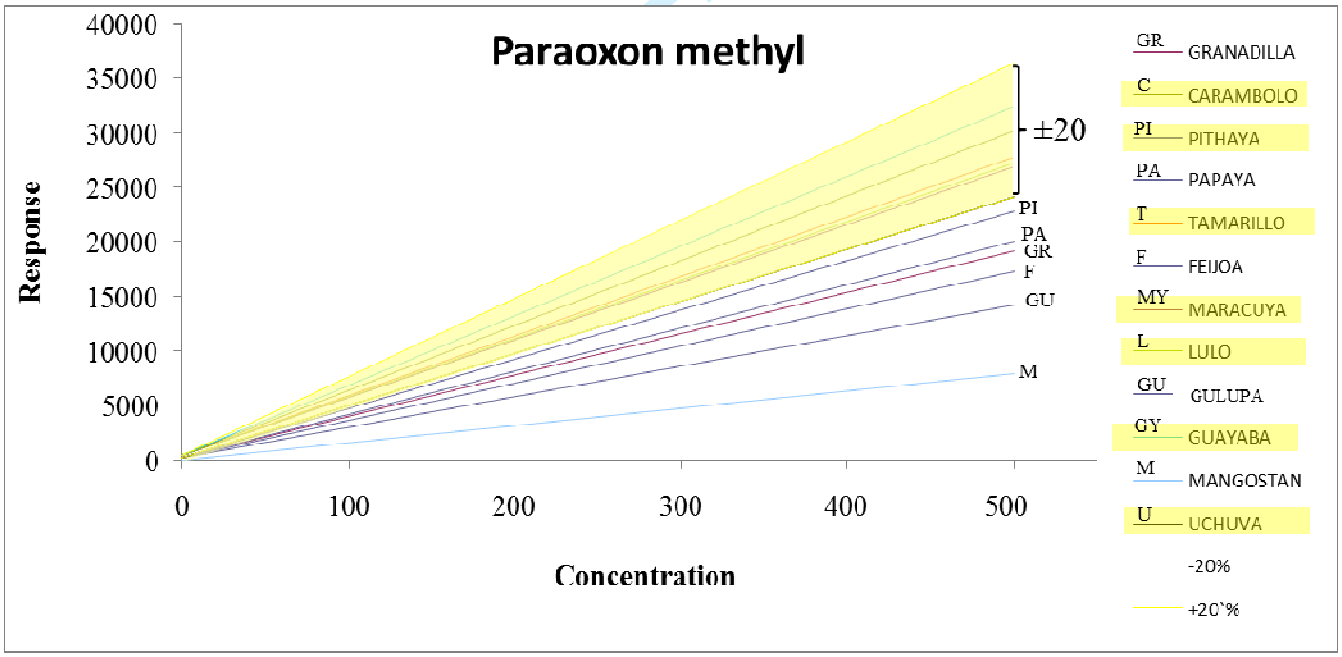

Clothianidin
Response
Concentration
17500
15000
12500
10000
7500
5000
2500
0
0
100
200
300
400
500
C
GY
M
GR
±20
GR GRANADILLA
C CARAMBOLO
PI PITHAYA
PA PAPAYA
T TAMARILLO
F FEIJOA
MY MARACUYA
L LULO
GU GULUPA
GY GUAYABA
M MANGOSTAN
U UCHUVA
-20%
+20%

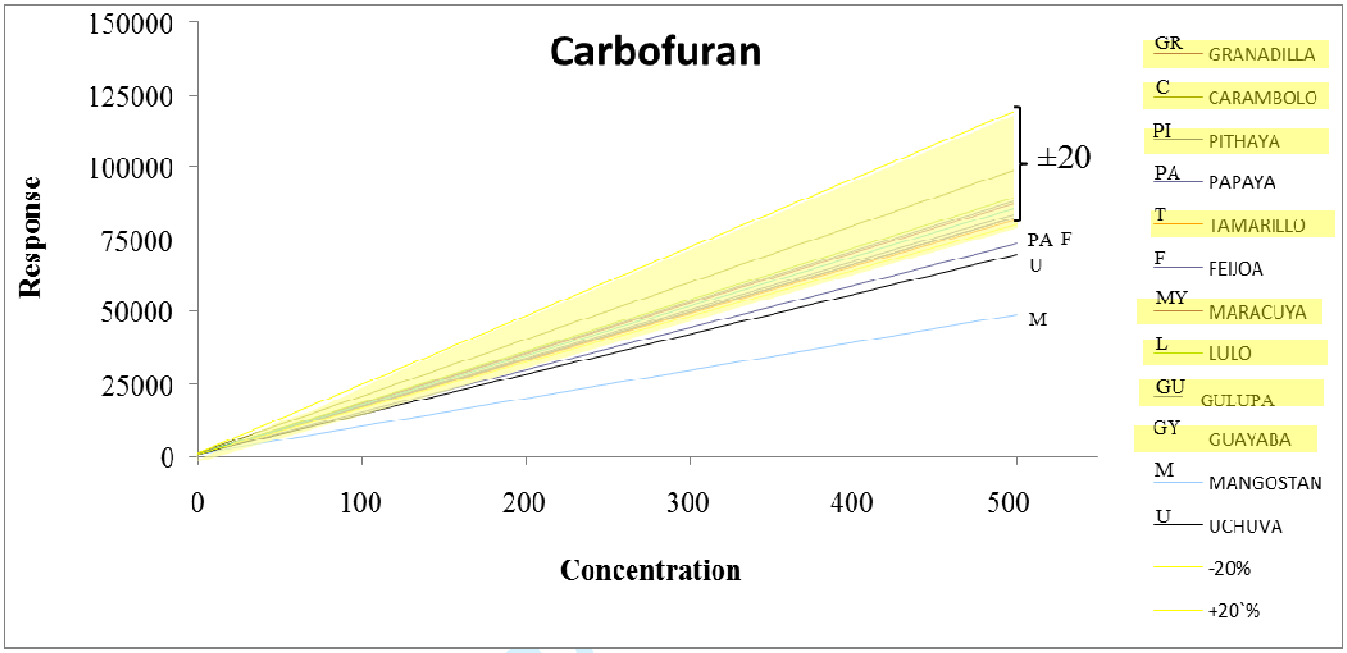

3 OH-Carbofuran
Response
Concentration
8000
7000
6000
5000
4000
3000
2000
1000
0
0
100
200
300
400
500
L
U
GY
T
M
C
MY
PA
±20
GR GRANADILLA
C CARAMBOLO
PI PITHAYA
PA PAPAYA
T TAMARILLO
F FEIJOA
MY MARACUYA
L LULO
GU GULUPA
GY GUAYABA
M MANGOSTAN
U UCHUVA
20%
+20%

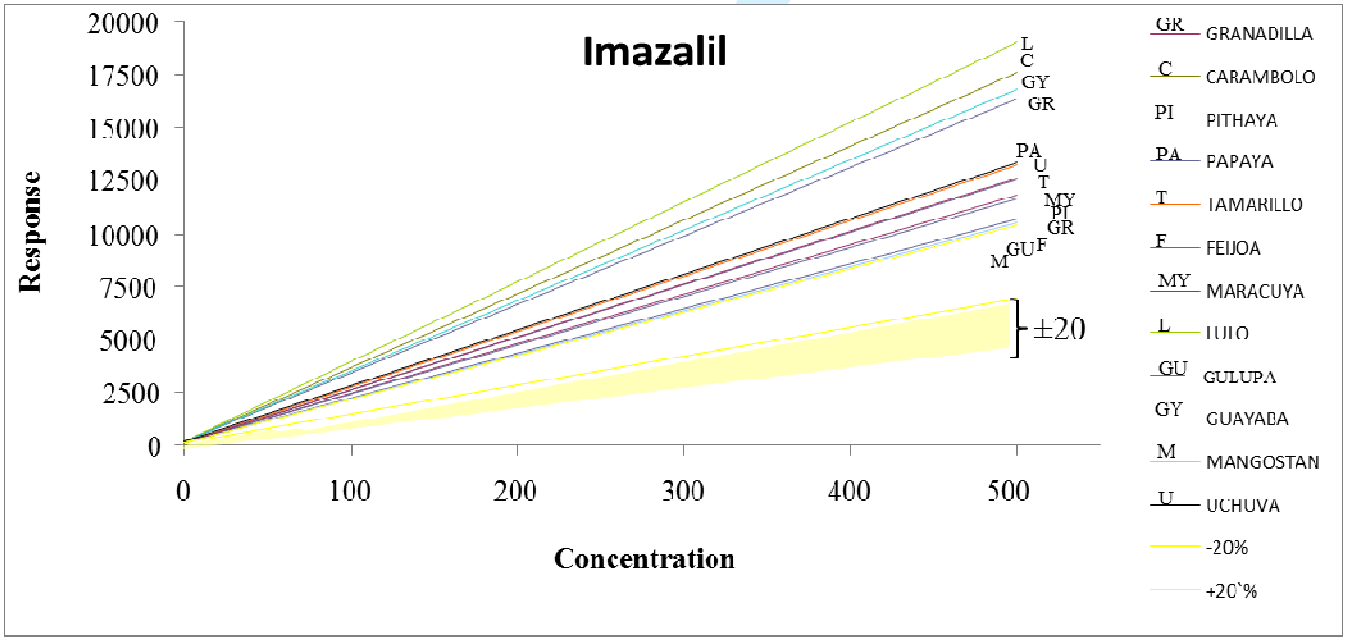

Dimethoate
Response
Concentration
30000
25000
20000
15000
10000
5000
0
0
100
200
300
400
500
L
±20
GR GRANADILLA
C CARAMBOLO
PI PITHAYA
PA PAPAYA
T TAMARILLO
F FEIJOA
MY MARACUYA
L LULO
GU GULUPA
GY GUAYABA
M MANGOSTAN
U UCHUVA
-20%
+20`%

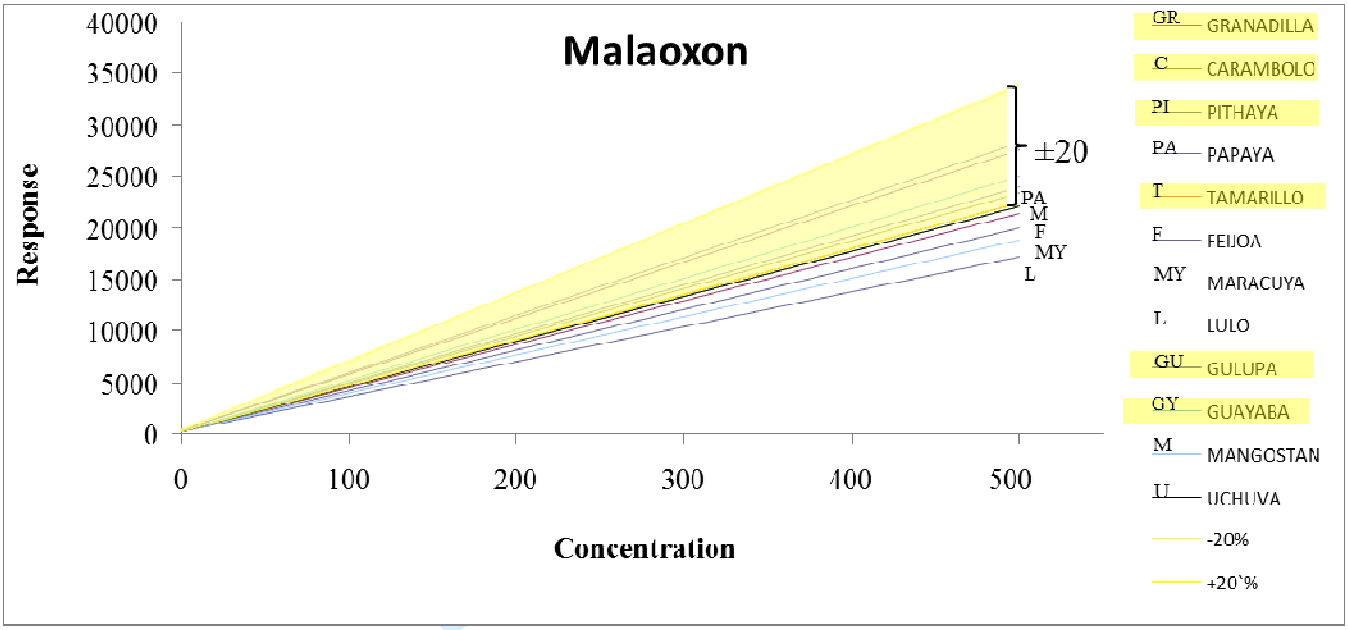

Thiabendazole
Response
Concentration
35000
30000
25000
20000
15000
10000
5000
0
0
100
200
300
400
500
C
PI
PA
T
GY
GR U
M
L
MY
GU
±20
GR GRANADILLA
C CARAMBOLO
PI PITHAYA
PA PAPAYA
T TAMARILLO
F FEIJOA
MY MARACUYA
L LULO
GU GULUPA
GY GUAYABA
M MANGOSTAN
U UCHUVA
20%
+20`%

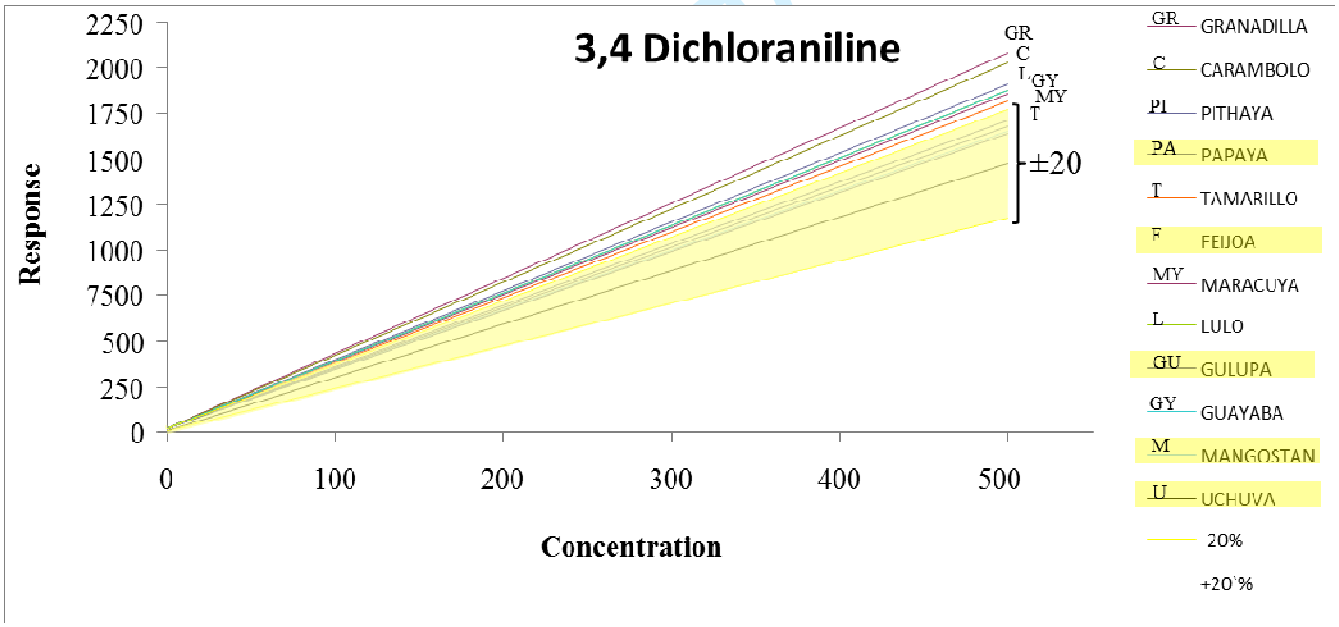

Thiacloprid
Response
Concentration
80000
70000
60000
50000
40000
30000
20000
10000
0
0
100
200
300
400
500
±20
C
GU
GR
F
M
GR GRANADILLA
C CARAMBOLO
PI PITHAYA
PA PAPAYA
T TAMARILLO
F FEIJOA
MY MARACUYA
L LULO
GU GULUPA
GY GUAYABA
M MANGOSTAN
U UCHUVA
-20%
+20`%

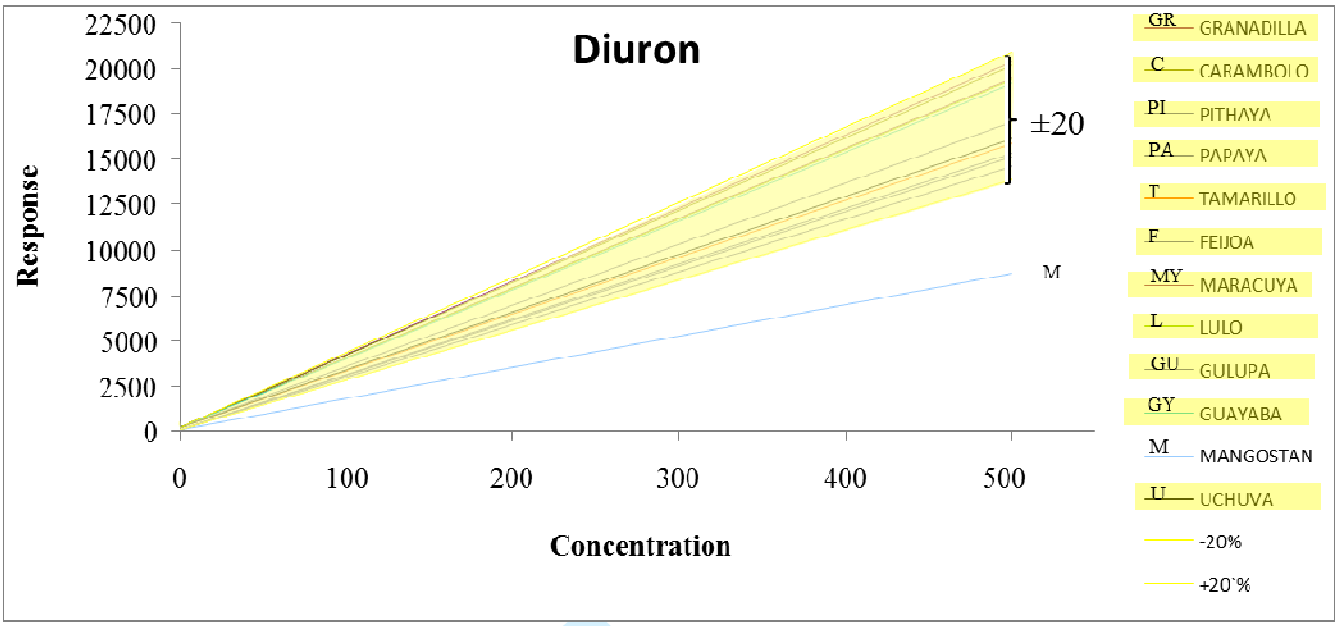

Paraoxon methyl
Response
Concentration
40000
35000
30000
25000
20000
15000
10000
5000
0
0
100
200
300
400
500
±20
PI
PA
GR
F
GU
M
GR GRANADILLA
C CARAMBOLO
PI PITHAYA
PA PAPAYA
T TAMARILLO
F FEIJOA
MY MARACUYA
L LULO
GU GULUPA
GY GUAYABA
M MANGOSTAN
U UCHUVA
-20%
+20`%

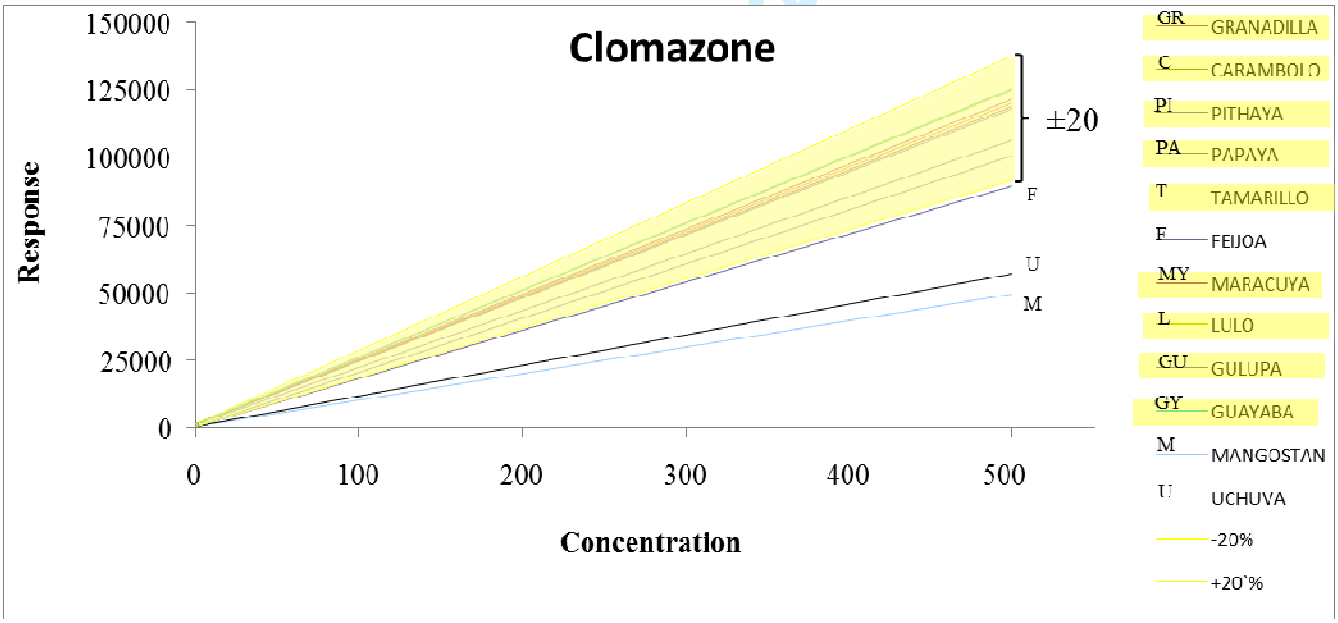

Carbofuran
Response
Concentration
150000
125000
100000
75000
50000
25000
0
0
100
200
300
400
500
±20
PA F
U
M
GR GRANADILLA
C CARAMBOLO
PI PITHAYA
PA PAPAYA
T TAMARILLO
F FEIJOA
MY MARACUYA
L LULO
GU GULUPA
GY GUAYABA
M MANGOSTAN
U UCHUVA
-20%
+20`%

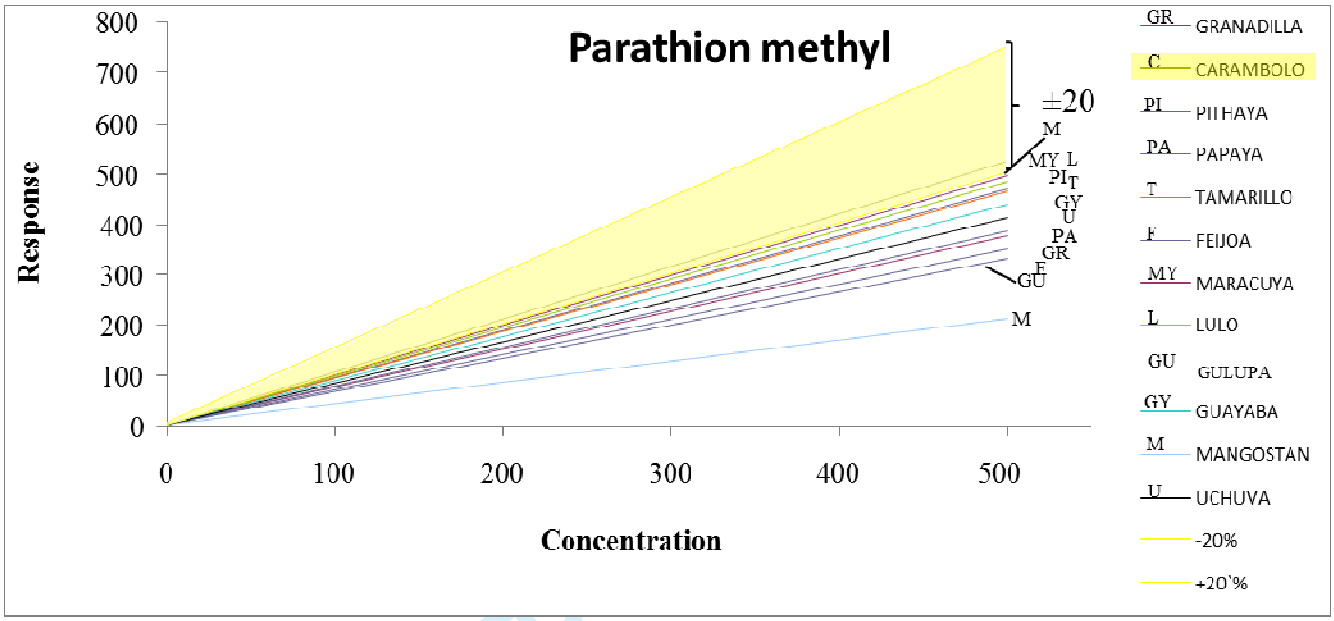

Imazalil
Response
Concentration
20000
17500
15000
12500
10000
7500
5000
2500
0
0
100
200
300
400
500
L
C
GY
GR
PA
U
T
MY
PI
GR
GU
F
M
±20
GR GRANADILLA
C CARAMBOLO
PI PITHAYA
PA PAPAYA
T TAMARILLO
F FEIJOA
MY MARACUYA
L LULO
GU GULUPA
GY GUAYABA
M MANGOSTAN
U UCHUVA
-20%
+20`%

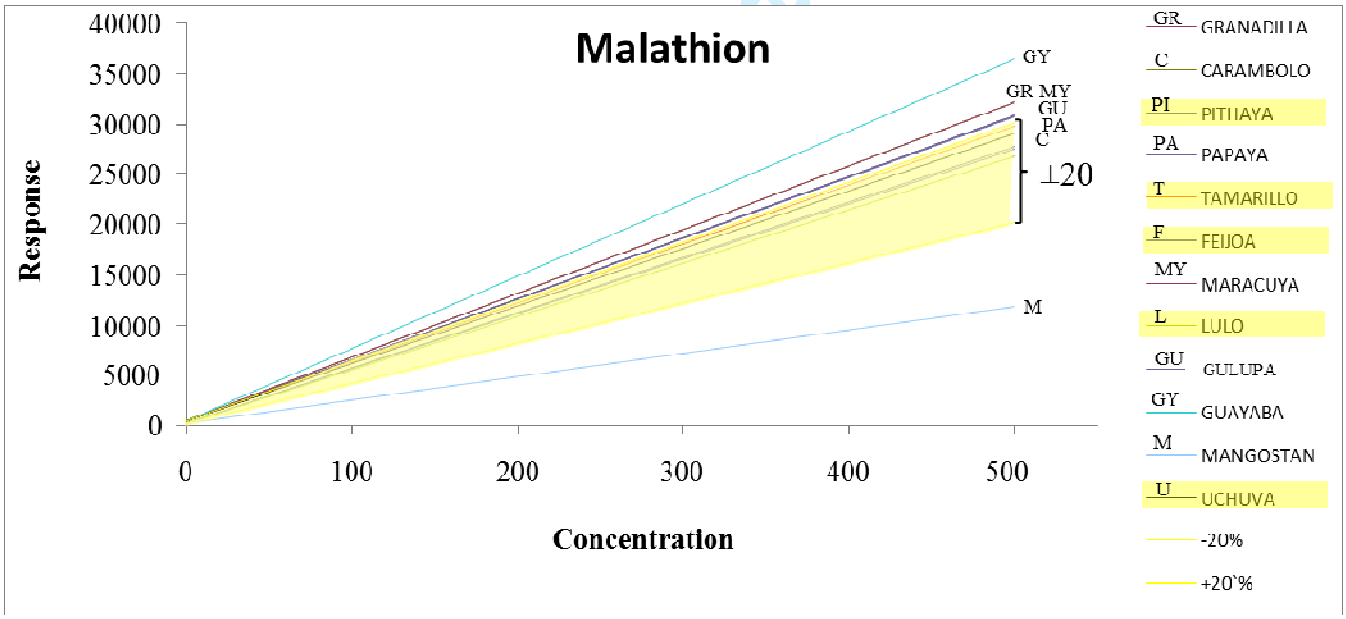

Malaoxon
Response
Concentration
40000
35000
30000
25000
20000
15000
10000
5000
0
0
100
200
300
400
500
±20
PA
M
F
MY
L
GR GRANADILLA
C CARAMBOLO
PI PITHAYA
PA PAPAYA
T TAMARILLO
F FEIJOA
MY MARACUYA
L LULO
GU GULUPA
GY GUAYABA
M MANGOSTAN
U UCHUVA
-20%
+20%

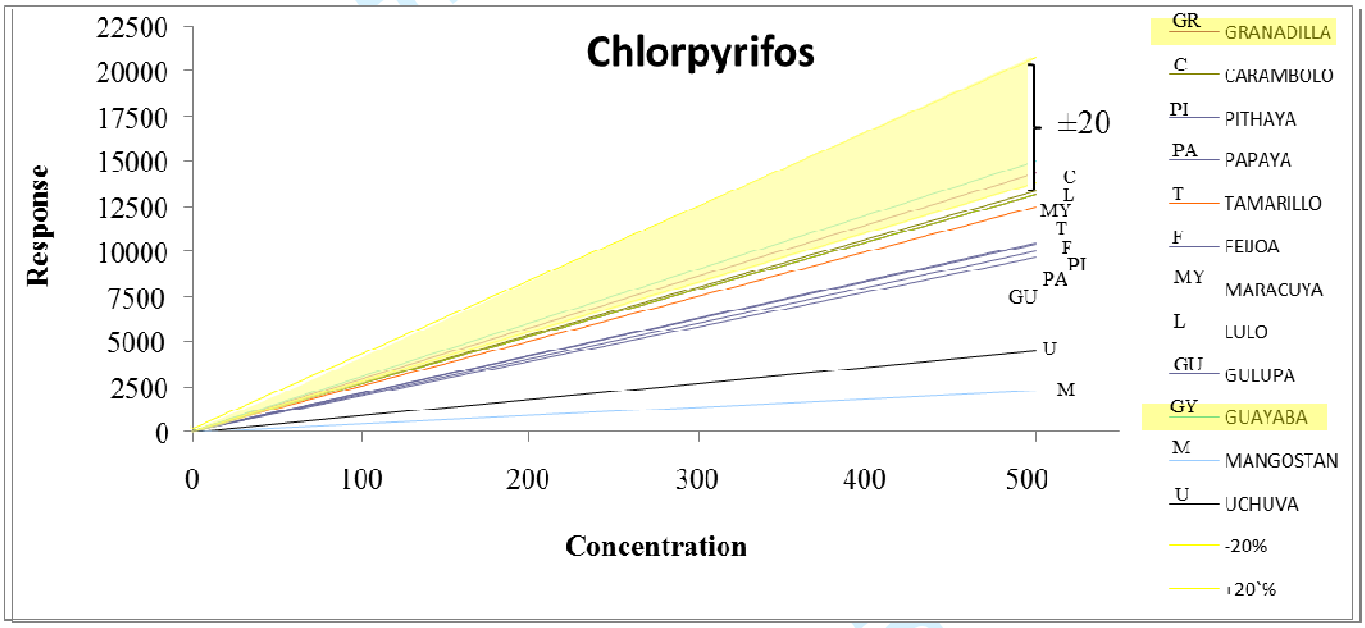

3,4 Dichloraniline
Response
Concentration
2250
2000
1750
1500
1250
1000
7500
500
250
0
0
100
200
300
400
500
GR
C
L
GY
MY
T
±20
GR GRANADILLA
C CARAMBOLO
PI PITHAYA
PA PAPAYA
T TAMARILLO
F FEIJOA
MY MARACUYA
L LULO
GU GULUPA
GY GUAYABA
M MANGOSTAN
U UCHUVA
20%
+20%


Diuron
Response
Concentration
22500
20000
17500
15000
12500
10000
7500
5000
2500
0
0
100
200
300
400
500
±20
M
GR GRANADILLA
C CARAMBOLO
PI PITHAYA
PA PAPAYA
T TAMARILLO
F FEIJOA
MY MARACUYA
L LULO
GU GULUPA
GY GUAYABA
M MANGOSTAN
U UCHUVA
-20%
+20%


Clomazone
Response
Concentration
150000
125000
100000
75000
50000
25000
0
0
100
200
300
400
500
±20
F
U
M
GR GRANADILLA
C CARAMBOLO
PI PITHAYA
PA PAPAYA
T TAMARILLO
F FEIJOA
MY MARACUYA
L LULO
GU GULUPA
GY GUAYABA
M MANGOSTAN
U UCHUVA
-20%
+20%


Parathion methyl
Response
Concentration
0
100
200
300
400
500
600
700
800
±20
M
MY L
PI
T
GY
U
PA
GR
F
GU
M
GR GRANADILLA
C CARAMBOLO
PI PITHAYA
PA PAPAYA
T TAMARILLO
F FEIJOA
MY MARACUYA
L LULO
GU GULUPA
GY GUAYABA
M MANGOSTAN
U UCHUVA
-20%
+20'%


Malathion
Response
Concentration
0
5000
10000
15000
20000
25000
30000
35000
40000
100
200
300
400
500
GY
GR MY
GU
PA
C
⊥20
M
GR GRANADILLA
C CARAMBOLO
PI PITHAYA
PA PAPAYA
T TAMARILLO
F FEIJOA
MY MARACUYA
L LULO
GU GULUPA
GY GUAYABA
M MANGOSTAN
U UCHUVA
-20%
+20'%


Chlorpyrifos
Response
Concentration
22500
20000
17500
15000
12500
10000
7500
5000
2500
0
0
100
200
300
400
500
±20
C
L
MY
T
F
PI
PA
GU
U
M
GR GRANADILLA
C CARAMBOLO
PI PITHAYA
PA PAPAYA
T TAMARILLO
F FEIJOA
MY MARACUYA
L LULO
GU GULUPA
GY GUAYABA
M MANGOSTAN
U UCHUVA
-20%
+20%