Journal of Experimental Biology and Agricultural Sciences, April - 2023; Volume – 11(2) page 339 – 350

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Determination of carbendazim residues in Moroccan tomato samples using local enzyme-linked immunosorbent assay and comparison with liquid chromatography

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Received – November 17, 2022; Revision – March 14, 2023; Accepted – April 27, 2023
Available Online – April 30, 2023

DOI: http://dx.doi.org/10.18006/2023.11(2).339.350

KEYWORDS

Carbendazim

Moroccan tomato

HPLC-UV

ELISA

Extraction

ABSTRACT

The fungicide carbendazim (CBZ) is not approved for agricultural uses in some countries but is still used by many farmers due to its effectiveness. For this reason, in previous work of the same authors, they developed a competitive enzyme immunoassay (ELISA) using rabbit polyclonal antibodies to detect CBZ. This study aimed to validate this in-house ELISA after extraction with methanol for CBZ analysis in tomato samples, and the results were compared with the conventional high-performance liquid chromatography (HPLC) method after QuEChERS extraction. The results showed that both ELISA and HPLC methods have good repeatability, reproducibility and high precision with a good variation verified by principal components analysis (PCA). ANOVA tested the detection limit (LOD), and quantification limit (LOQ), and the values for ELISA (LOD = 0.026± 0.001 µg/L and LOQ = 0.083 ± 0.003 µg/L) were significantly lower than those obtained by HPLC (LOD = 0.61 ± 0.02 µg/L and LOQ = 1.85 ± 0.07 µg/L). ELISA and HPLC were used for analyzing CBZ in 100 Moroccan tomato samples. These two methods detected the presence of CBZ above the Maximum Residue Limit (MRL) level in 9 samples. However, the presence of the CBZ was detected in the 79 samples by ELISA and quantified in 66 samples. In contrast, the presence of CBZ was detected in 57 and quantified in 35 samples by HPLC. These results showed that the ELISA system coupled with a simple methanol extraction is much more sensitive than HPLC after QuEChERS extraction.

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI] (http://www.horizonpublisherindia.in/).

1 Introduction

Pesticides play an important role in the development of agriculture by reducing agricultural product losses, improving productivity, controlling vector diseases and contributing to the diversification of food (Patibanda and Ranganathswamy 2018). In 2019, global pesticides used in agriculture crop protection was around 4.2 million tons of active ingredients, and worldwide pesticide application was 2.7 kg/ha per cropland area (FAO 2021). Morocco is an agricultural economy country and produces various crops every year, requiring large quantities of pesticides. Due to the absence of a national phytopharmaceutical industry, Morocco imported around 22,000 tonnes of pesticides in 2019 (Bouterfas et al. 2022).

Some pesticides are potentially hazardous and can cause harm to animal and human health and the ecosystem when they persist in the environment (Sharma et al. 2019; Sarkar et al. 2021). Different concentrations of pesticides have been found in various food samples (Moussaif et al. 2021), and it has been shown that the dietary intake containing pesticides represents the primary source of exposure in humans leading to neurological and developmental disorders (Sakali et al. 2021). Several works have shown possible links between pesticide consumption and various health effects such as cardiovascular disease, reproductive disorders, Parkinson's disease, etc. (Nicolopoulou-Stamati et al. 2016). In addition, these chemicals are suspected to have neurotoxic, cytotoxic, genetic mutation, chromosomal and DNA damage effects (Barron Cuenca et al. 2022).

According to the Food and Agriculture Organisation statistics (FAOSTAT), fungicides are the second most used pesticides, with 31% of world consumption after insecticides (FAOSTAT 2022). Fungicides protect crops against harmful fungi that cause several plant diseases. They also protect agricultural products during storage (Magunga and Malebo 2023). Methyl 2-benzimidazole carbamate or carbendazim (CBZ) is a benzimidazole fungicide that has been widely used to treat soils and protect many crops such as cereals, fruits and vegetables against fungal pathogens (Wang et al. 2023). However, CBZ is known to be hazardous for humans, animals and the environment (Kasaeinasab et al. 2023). It has been banned in Australia, USA, and most European Union countries (Elshafey et al. 2022). In Morocco, CBZ was registered and approved by ONSSA (National Office of Food Safety) for eight crops, including onion, tomato, apricot, peach, pear, apple, vine and rose before 2017. The fungicide has been banned by ONSSA since 2017, but farmers are still using formulations containing carbendazim, accounting for approximately 40% of all pesticide formulations used in agriculture in 2021 (Ben Khadda et al. 2021).

Solanum lycopersicum L. (tomato) is a trendy vegetable crop, and it is widely consumed worldwide. Along with many beneficial nutrients, tomatoes are also known to reduce the effects of chronic diseases, cancer, cardiovascular diseases, osteoporosis etc. (Kumar et al. 2020). In Morocco, tomatoes are economically important crops and rank second in the position of exported crops after citrus. In 2018, Morocco became the world's 15th tomato producer, producing more than 1,409.44 million fresh tomatoes and a yield of 8.83 kilos per metre of cultivated land (FAOSTAT 2022). Improving this productivity has often been associated with the action of pesticides (Benaboud et al. 2021). It should be noted that CBZ is applied by spraying in the field or by dipping produce after harvest. It is used in high doses and often repeated as per the target disease (Wang et al. 2012). Recently, its residues have been detected in various Moroccan tomato samples (Choubbane et al. 2022).

Many analytical techniques, such as high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), gas chromatography (GC), etc., are used for the analysis of carbendazim residue (Lesueur et al. 2008; Phansawan et al. 2015). However, conventional methods are slow, expensive and require several solvents, especially when analyzing many samples (Aylaz et al. 2021). Therefore, other techniques like immunoassays could be exciting alternatives to chromatography. These techniques often detect single analytes with high specificity (Liu et al. 2007). Enzyme-linked immunosorbent assay (ELISA) has been used to analyze several pesticides (Maftouh et al. 2020). In a previous work by Bellemjid et al. (2018), indirect competitive ELISA was developed for CBZ detection.

This study aimed to validate the in-house ELISA system for CBZ analysis in tomatoes after a simple extraction procedure and to apply this system to detect the fungicide in 100 tomato samples purchased from local Moroccan markets. The results were compared with the well-established HPLC method after QuEChERS extraction.

2 Material and methods

2.1 Sampling methodology and preparation

One hundred tomato samples with an average weight of 80 to 100g were collected from the market in the province of Rabat, Morocco, from January to June 2022. An organic tomato sample from the bio-market was used for extraction optimization and control. A representative portion of each tomato sample was blended, and aliquots of 10 g were stored at -20 °C.

2.2 Extraction of CBZ

Figure 1 illustrates the schematic flowchart of extractions methods used for HPLC and ELISA analysis. For HPLC analysis, the extraction of CBZ from blended tomato samples was made according to the QuEChERS method (Quick, Easy, Cheap, Effective, Rugged and Safe method) (Kim et al. 2019). A

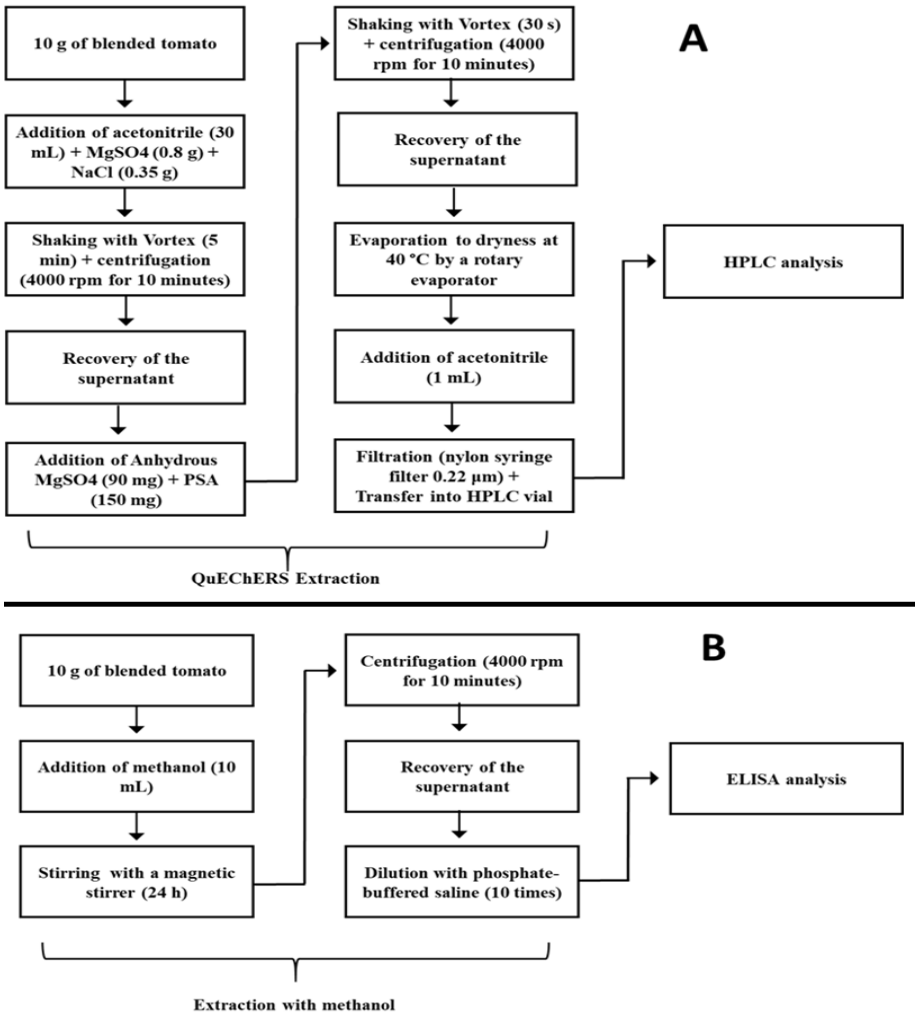


Figure 1 Schematic presentation of CBZ extractions methods in tomato samples. A: QuECheRS method used for HPLC analysis. B: methanol extraction used for ELISA analysis.

total of 10 g of blended tomato was mixed with 0.8 g of $MgSO_4$ and 0.35 g of NaCl in 30 mL of acetonitrile (ACN), and the mixture was shaken for 5 minutes. This was followed by centrifuging the mixture for 10 minutes at 4000 rpm at 4°C. Anhydrous $MgSO_4$ (90 mg) and PSA (150 mg) were added to the supernatant. After shaking for 30 seconds, the mixture was centrifuged at 4000 rpm for 5 minutes. A rotary evaporator evaporated the supernatant to dry at below 40 °C. After that, 1 mL of acetonitrile was added to the dry extract, and the mixture was vortexed and transferred to an HPLC vial after filtration with a 0.22 µm nylon syringe filter.

For ELISA analysis, tomato extracts were prepared by mixing 10 g of tomato samples with 10 ml of methanol and shaking for 24 hours. After centrifugation at 4000 rpm for 10 minutes, the supernatant was collected and diluted 10-fold with phosphate-buffered saline (PBS).

2.3 ELISA development and analysis

A rabbit polyclonal antibody ELISA test for the detection of CBZ was developed as described in previous work by the same authors (Bellemjid et al. 2018). Figure 2 illustrates the development phases of the ELISA test.

Briefly, 2 CBZ haptens with 4 or 5 carbon spacer arms were synthesized. A hapten with four carbon spacer arms has been conjugated to bovine serum albumin (BSA) and used for antibody production in rabbits. The 5-carbon Hapten is conjugated to human albumin (HAS) and coated in well plates. After that, the coating antigen, antibody dilution and standard concentration were optimized. For the indirect ELISA test, CBZ was 100 ng/well hapten HSA as coating, 1/2000 of antiserum anti-CBZ as a diluent and 0.01 to 1000 µg/L CBZ as standard curve (Bellemjid et al. 2018).

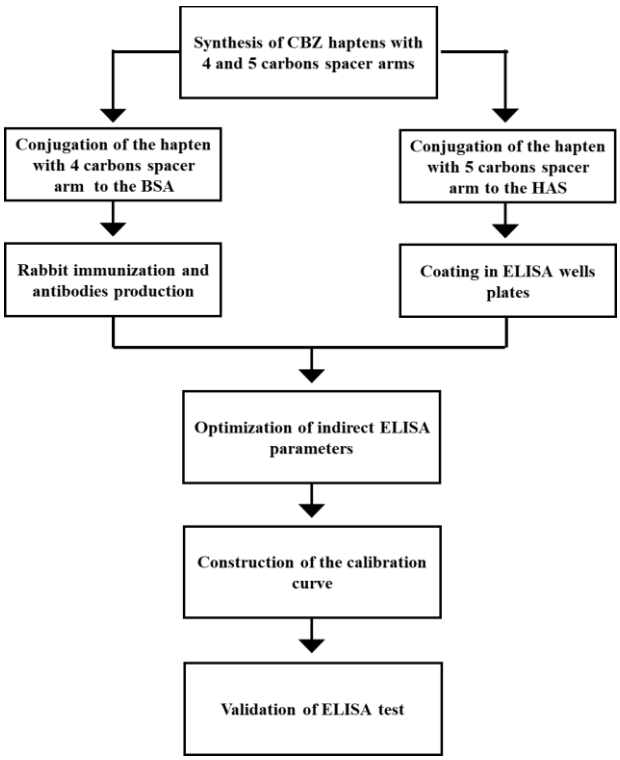


Figure 2 Schematic presentation of ELISA development.

For the immunoassay test, hapten-HSA was coated on microtiter plates using 50 mM carbonate-bicarbonate at pH 9.6 for 12 hours at 4°C. After washing with PBS, the wells were blocked with 300 µl of 3% skim milk in PBS for 12 hours at 4°C. Wells were washed twice with PBS, and 50 µl of CBZ standards in PBS containing 10% methanol were added to each well, followed by 50 µl of 1/2000 v/v antiserum in PBS. After incubation at 28°C for 2 hours, the plate was washed 5 times with PBS (PBST) containing 0.05% Tween 20, then 100 µl of peroxidase-conjugated anti-rabbit IgG was added to each well and incubated at 28°C for 1 hour. The plate was re-washed 5 times with PBST and 100 µl/well OPD (1mg/mL of O-phenylenediamine dihydro-chloride in citrate-acetate buffer pH 5.5 and containing 0.1% of H_2O_2). The reaction was stopped by adding 50 µl of 3 M HCl, and the absorbance was measured at 492 nm. The following formula allows the calculation of relative binding (Maftouh et al. 2020):

$$\%\frac{B}{B_0} = \frac{A - A_{XS}}{A_0 - A_{XS}} \times 100$$

A: the absorbance. A_0: the zero-dose absorbance of the CBZ.A_{xs}: the absorbance to an excess of the CBZ.

A standard curve that looks like a sigmoid was constructed by plotting the B/B0 value (%) versus the logarithm of the analyte concentration (Raab 1983). SigmaPlot® 14.0 software (Systat Software, San Jose, CA, USA) was used to plot the ELISA competition curve for CBZ. The IC_{50} value (50% inhibition of antigen/antibody binding) was calculated using the following function (Rodbard 1981):

$$y = D + \frac{A - D}{1 + 10^{B(X - \log C)}}$$

A: the minimum percentage of B/B_0 (representing an infinite concentration of CBZ), D: $\%B/B_0$ at the maximum (zero concentration of CBZ), Log C: the logarithm of the IC_{50} corresponding to the inflexion point of the curve, B: the Hill's slope at C, X: the concentration of free CBZ

For CBZ analysis in samples, 50 µL/well of diluted methanol extract was used in the immunoassay system (as described above). The concentration values were obtained by fitting the sigmoidal curves. The fungicide concentrations were reported to mg/kg of fresh tomato, considering the extraction dilutions.

2.4 Cross-reactivity in ELISA analysis

The ELISA system was prepared with a standard curve of the studied compounds to evaluate the percentage of cross-reactivity (CR) with other pesticides structurally related to CBZ. The CR is the ratio of the CBZ IC_{50} to the IC_{50} of the tested compound and is expressed as a percentage CR. Benzimidazole (benomyl, Thiabendazoleand fuberidazole) and carbamate (Carbaryl and carbofuran) pesticides were tested in the developed ELISA system.

2.5 HPLC analysis

The stock solution of the certified CBZ (Honeywell Fluka analytical standards Munich, Germany) was prepared at 1 mg/mL in acetonitrile and stocked in the dark at 4 °C until use. Standard solutions of CBZ (0.0001, 0.001, 0.025, 0.05, 0.1 and 1 mg/L) were prepared independently in acetonitrile from the stock solution. HPLC analysis was performed by Alliance Waters 2695 system. The column was a Sunfire C18 column (4.6 mm x 250 mm, 5 µm, 100Å). The mobile phase consisted of acetonitrile/acidified water containing 0.1% phosphoric acid (20/80, v/v), with a 1.0 mL/min flow rate and 10 µL injection volume. The detection wavelength was 285 nm, and analyses were performed at 25°C. The calibration curve has been plotted and corresponds to the peak area versus CBZ concentration. Its linearity was verified by the correlation coefficient (r^2).

2.6 Evaluation of recovery percentages

Recovery studies were evaluated by adding known concentrations of CBZ (0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 mg/kg) to mixed organic tomatoes. The mixture was shaken at room temperature for one hour. Methanol extracts and QuEChERS extracts were analyzed using the developed immunoassay and HPLC, respectively. The accuracy of the method was determined by analyzing a blank (organic tomato) and a spiked blank. The percent recovery of CBZ was calculated using the following formula (Moussaif et al. 2021):

$$\% \text{ Recovery} = \frac{\text{Amount of pesticide after spiking}}{\text{Original pesticide content + Spiked amount}} \times 100$$

2.7 Statistical data analysis

Limits of detection (LOD), limits of quantitation (LOQ), precision and repeatability parameters were determined according to Boscolo et al. (2013) LOD is the lowest concentration that gives a response equal to 3 times the baseline noise, and it was determined from the analysis of the 0 standards. The LOQ is the lowest fungicide concentration that gives a response 10 times the baseline noise. The precision of the analytical method was assessed by repeating and reporting the relative standard deviation (RSD%). Data were analyzed using IBM SPSS statistics software, version 27.0. The validation parameters of ELISA and HPLC methods are subjected to principal components analysis (PCA) to evaluate the variation. Values were expressed as mean ± standard deviation (SD) of 5 independent assays. The comparison was carried out by the analysis of variance (ANOVA), and significant mean differences were separated using the Tukey test at the 5% probability level.

3 Results

3.1 Optimisation Indirect ELISA for CBZ analysis and performances

The indirect ELISA was developed using 100 ng/well of antigen coating and 1/2000 antibodies dilution. These antibodies were used for competition between the free CBZ and the corresponding coated CBZ-hapten conjugate. A typical standard curve of CBZ immunoassay is shown in Figure 3. The IC_{50} was recorded at 0.63 ± 0.024µg/L during this study.

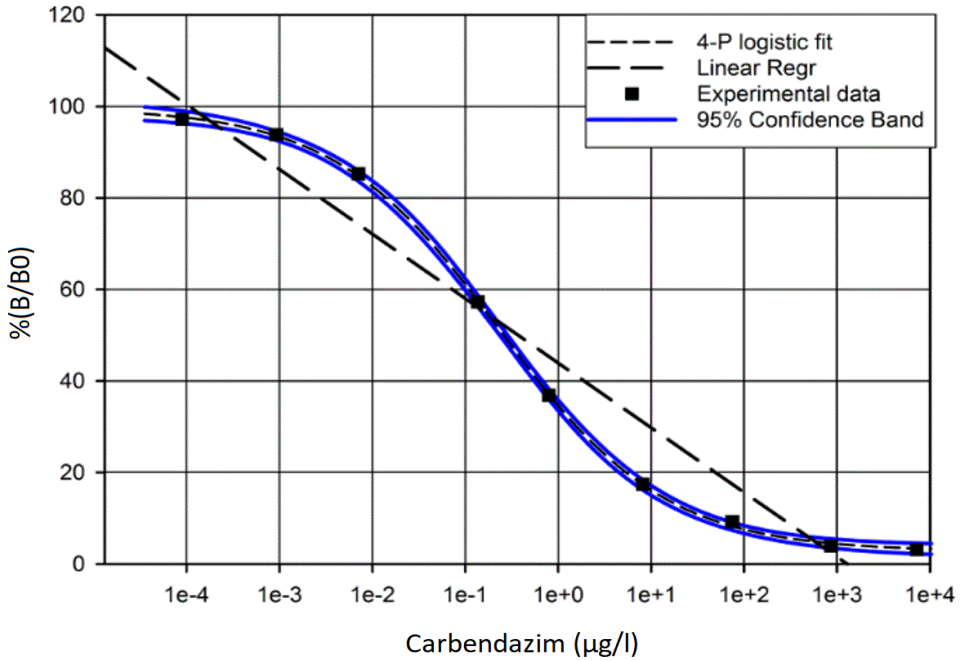


Figure 3 Indirect immunoassay standard carbendazim (CBZ) curves using 100 ng of CBZ-HAS for coating and anti-CBZ at 1/2000. Serial dilutions of the CBZ (0.01 to 1000 µg/L) were used as standards

The characteristics of the designed ELISA system are regrouped in Table 1. The results showed good repeatability (RSD = 4.35 %) and reproducibility (RSD = 1.22 %). LOD and LOQ values were recorded 0.026± 0.001 µg/L and 0.083 ± 0.003 µg/L respectively. Higher accuracy was obtained with a percentage recovery from 88.1 ± 4.3 to 96.9 ± 1.9 % (Table 1). It should be noted that the resistance of the ELISA to methanol used to dissolve CBZ was tested, and the results showed that the inhibition of reactivity by more than 10% methanol in PBS increases with increasing solvent concentration (for example, when methanol concentrations were 15 and 20%, the IC_{50} values increased by 1.5 and 1.9 fold respectively).

Table 1 Validation of CBZ analysis using ELISA and HPLC-UV

Validation parameters	ELISA	HPLC
Correlation coefficient (r^2)	-	0.998
Detection limit (LOD) (µg/L)	0.026 ± 0.001 [a]	0.61± 0.02 [b]
Quantification limit (LOQ) (µg/L)	0.083 ± 0.003 [c]	1.85 ± 0.07 [d]
Repeatability (RSD%)	4.35	3.18
Reproducibility (RSD%)	1.22	0.19
Accuracy at 0.05 mg/kg (% recovery)	95.6 ± 2.5	96.2 ± 2.2
Accuracy at 0.1 mg/kg (% recovery)	96.9 ± 1.9	91.3 ± 3.6
Accuracy at 0.2 mg/kg (% recovery)	88.1 ± 4.3	97.4 ± 1.2
Accuracy at 0.3 mg/kg (% recovery)	89.5 ± 3.2	98.1 ± 1.3
Accuracy at 0.4 mg/kg (% recovery)	91.7 ± 4.5	85.7 ± 4.2
Accuracy at 0.5 mg/kg (% recovery)	95.2 ± 2.8	89.1 ± 4.6

Given values are the average of five replicates; values followed by ± are SD (Standard Deviation); Different superscript letters indicate significant differences in LOD and LOQ between ELISA and HPLC

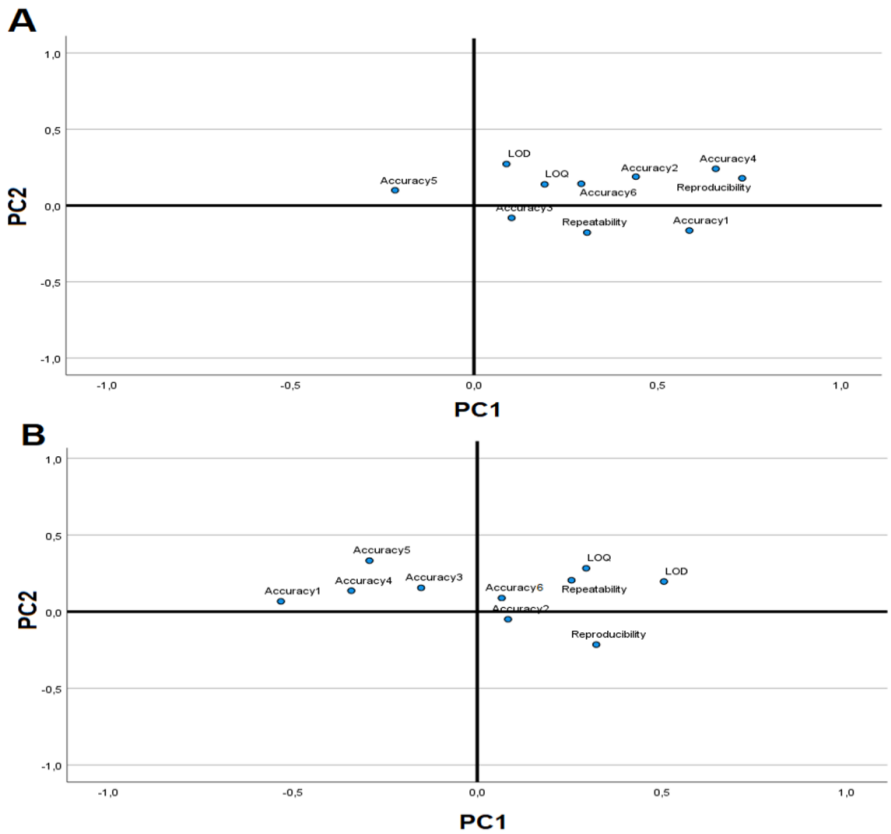


Figure 4 Principal component analysis (PCA) for validation parameters of ELISA (A) and HPLC (B) analytical methods

Principal component analysis (PCA) was used to understand the variability of the validation parameters in five different assays. The validation parameters (LOD, LOQ, repeatability, reproducibility, accuracy1 at 0.05 mg/kg, accuracy 2 at 0.1 mg/kg, accuracy 3 at 0.2 mg/kg, accuracy 4 at 0.3 mg/kg, and accuracy 5 at 0.4 mg/kg and accuracy 5 at 0. 5 mg/kg) collection points were the variables, and number of tests (5 tests) were the matrix lines. The two principal components explained 99.63 % and 98.84 % of variability for all variables in the data for ELISA and HPLC, respectively. The data set was visualized in the component shown in Figure 4.

The percentage CR of different compounds in the developed ELISA system was compared to CBZ and are reported in Table 2. Except for benomyl, the interference between CBZ and all tested compounds was negligible. The benomyl fungicide represented a high cross-reactivity, which CR percentage was around 83.1% (Table 2).

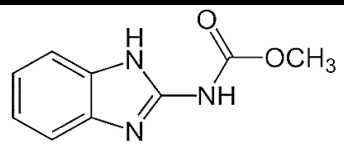
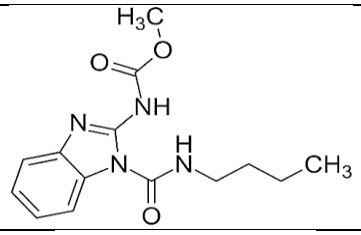
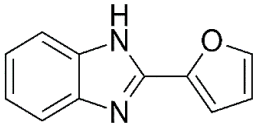
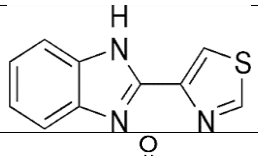
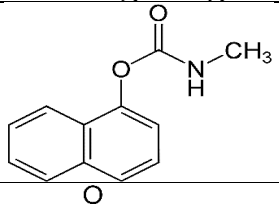
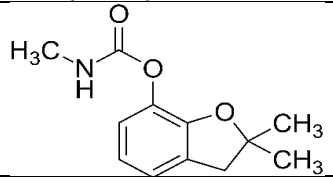
3.2 Validation of HPLC-UV method

Analytical data from the CBZ standards allowed statistical assessment of the accuracy and precision of the HPLC analysis. Validation parameters showed that the HPLC method could detect 0.61 ± 0.02 µg/L CBZ with a limit of quantitation (LOQ) of 1.85 ± 0.07 µg/L (Table 1). The correlation coefficient is 0.998, and the corresponding curve has good linearity and high accuracy (85.7 ± 4.2 to 98.1 ± 1.3 % recovery rate).

3.3 Correlation between ELISA and HPLC

Until the analysis of samples, analytical results for ELISA and HPLC using spiked organic tomatoes with CBZ (0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 mg/kg) were compared. The enriched samples estimated by ELISA and HPLC were close to the theoretical values. A good correlation was found between the two techniques, with a correlation coefficient (r2) of 0.994 (Figure 5).

Table 2 Cross-reactivity (CR) related to CBZ in the ELISA system

Compounds	Structure	%CR
CBZ		100
Benomyl		83.1
Fuberidazole		2.05
Thiabendazole		1.22
Carbaryl		0.63
Carbofuran		0.34

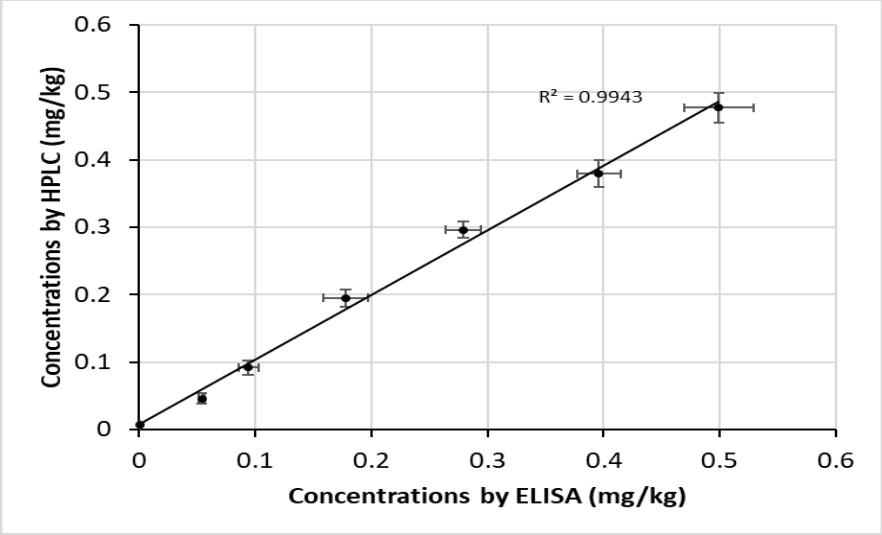


Figure 5 Comparisons of analytical results obtained for ELISA and HPLC using spiked organic tomato

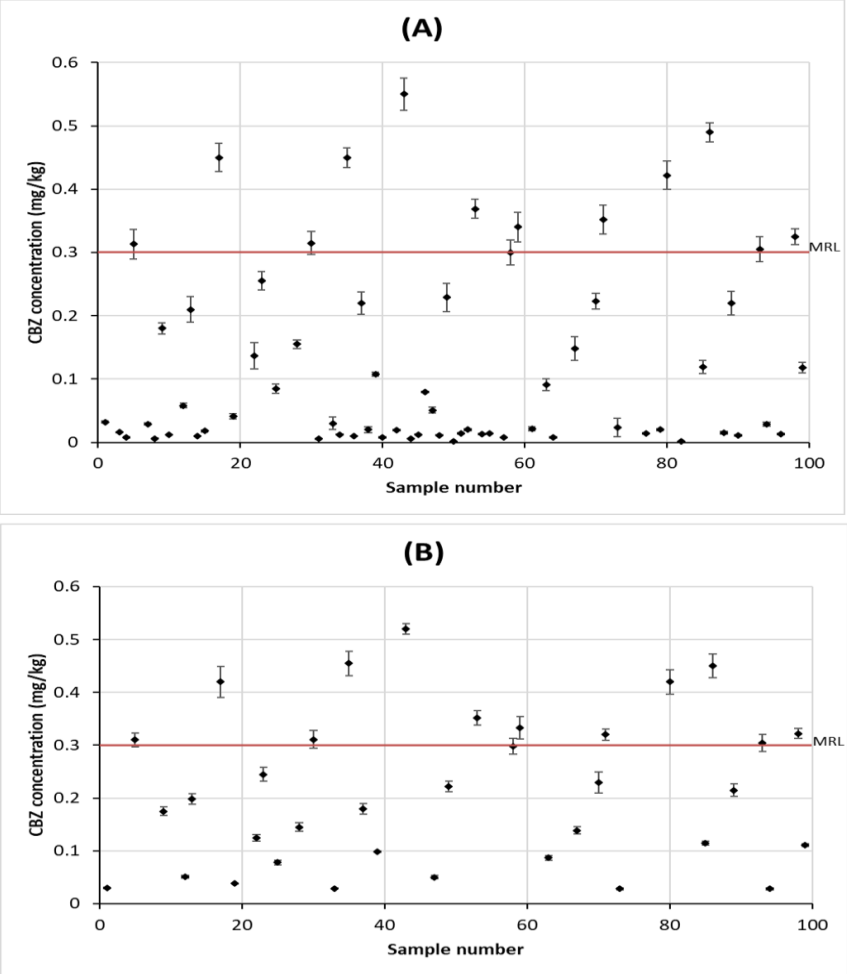


Figure 6 CBZ levels found in Moroccan tomato sample extracts (A) ELISA analysis, (B) HPLC analysis; Only quantified concentrations are represented. MRL (Maximum Residue Limit) are represented in the Figure, according to ONSSA 2014

Table 3 Parameters for the analyzed tomato samples

	ELISA	HPLC
Total samples	100	100
Total quantified	66	35
Total detected	79	57
Not detected	21	43
> MRL	9	9

3.4 CBZ analysis in tomato samples

The ELISA and HPLC systems were used to quantify the fungicide (CBZ) in 100 actual tomato samples. Figure 6 represents CBZ concentrations in samples that are statistically above the limit of quantification. The HPLC made quantifying about 34 among the 100 analyzed samples possible. These concentrations are approximately the same as found by the ELISA method (Figure 6). However, the developed ELISA quantified about 66 samples.

The results of both HPLC–UV and ELISA methods were compared, and the main parameters are included in Table 3. Based on three independent assays, both data sets show a statistical difference. In 100 analyzed samples, CBZ was detected in 57 by HPLC (> LOD), with 35 quantified samples (>LOQ) and 43 samples where CBZ was not detected. However, the developed ELISA detected the presence of CBZ in 79 samples, and it was quantified in 66 samples, and only 21 samples were without fungicide. Both methods detected the presence of CBZ above the maximum residue limit (MRL) in 9 samples (Figure 6 and Table 3). This determination of MRL was established by ONSSA (2014).

4 Discussion

Carbendazim (CBZ) has been used as a pre-and post-harvest treatment to control fungal diseases of various vegetables and fruits such as tomatoes (Liu et al. 2023). Because of its hazardous effects on humans, animals and the environment, the usage of CBZ in agricultural practices has been banned in Europe, Australia, USA and other countries, however, it is still used in some developing countries (Elshafey et al. 2022). For this reason, developing a rapid, sensitive assay to detect traces of CBZ in food is necessary. Immunoassays can meet these requirements, and several ELISA systems were used for detecting CBZ in different matrices (Yan et al. 2015; Liu et al. 2021). The characteristic of each ELISA is determined by the specificity and sensitivity of antibodies used to capture the target molecule.

Consequently, antibodies production strategy is essential for the immunoassay (Wu et al. 2022). In the previous work of Bellemjid et al. (2018), the production strategy of anti-CBZ was described. Hapten (4 carbons) of CBZ have been synthesized, coupled to BSA, and used to produce polyclonal antibodies in rabbits. On the other hand, Hapten(5 carbons) associated with HAS have been used for wells plates coating. For the indirect ELISA development, the antigen coating concentration and the antibodies dilution were optimized (100 ng/well for the antigen concentration and 1/2000 for the antibodies dilution). Antibodies were used for competition between standards (free CBZ) and the coated CBZ-hapten conjugate. A standard curve of CBZ immunoassay showed that IC_{50}, LOD and LOQ were 0.63 ± 0.024, 0.026 ± 0.001and 0.083 ± 0.003 µg/L respectively, with good repeatability (RSD = 4.35 %) and reproducibility (RSD = 1.22 %). In comparison, the direct ELISA system for detecting CBZ was performed by Song et al. (2019), with an IC_{50} value of 2.7 ± 0.3 µg/L and a LOD of 0.3 ± 0.15 µg/L. ELISA tests for CBZ using monoclonal antibodies were developed by Yan et al. (2015) with an IC_{50} value of 0.45 µg/L. This study validated the developed ELISA for CBZ analysis in tomatoes. Using ELISA to detect pesticides in food samples requires a prior extraction with organic solvents; generally, a simple extraction method is coupled to ELISA for the analysis (Verdini and Pecorelli 2022). In this study, a simple extraction with methanol was used to extract CBZ from tomato samples. The methanol extract was used for ELISA after 10 times dilution because beyond 10% of the reactivity of the antibodies decreased (Maftouh et al. 2020). In addition, the quality of the immunoassay is generally affected by interference and cross-reactions (CRs) (Maftouh et al. 2020). So, various pesticides were tested for CR in the developed ELISA. A higher CR of 83.1 % with benomyl was observed. Because of the tremendous structural similarity of benomyl with CBZ, the antibody has good reactivity. It should be noted that the degradation of benomyl obtains CBZ (Sebastian et al. 2022). The developed ELISA was used to detect CBZ in tomato samples collected from the market in the province of Rabat, Morocco. The results obtained by ELISA were compared with HPLC. After the QuEChERS extraction method, the HPLC analysis has been widely used for the CBZ analysis in water, soil, vegetables, etc. (Scheel and Tarley 2020). The QuEChERS method has been used intensely to extract various environmental pollutants and pesticides from different matrices (Kim et al. 2019). The HPLC method was validated for CBZ analysis with LOD and LOQ of 0.61 ± 0.02 and 1.85 ± 0.07 µg/L, respectively. These values are comparable with those found in the literature (Kim et al. 2019).

Nevertheless, ANOVA tested the LOD and LOQ for ELISA and HPLC, and the values obtained by HPLC were significantly higher than those obtained for ELISA. Several ELISAs developed for determining pesticides have shown lower LOD and LOQ values than HPLC (Zhai et al. 2023). The recovery of CBZ using methanol extraction followed by ELISA or QuEChERS extraction followed by HPLC analysis ranged from 85.7 to 98.1 % in the spiked tomato samples. These two selected methods presented a reasonable accuracy. The variability of all validation parameters was verified by principal component analysis (PCA).

ELISA and HPLC were used to analyze 100 tomato samples. HPLC and ELISA gave the same analytical results when CBZ was detectable and quantifiable. The two methods detected the presence of CBZ above the MRL level (0.3 mg/kg of tomato) in 9 samples. This MLR was recommended by the ONSSA in Morocco (ONSSA 2014). On the other hand, ELISA could quantify CBZ in 66 samples compared to HPLC (35 samples). In addition, ELISA's detection limit (LOD) was lower, allowing the detection of CBZ in 79 samples compared to HPLC (57 samples). These results showed an outstanding sensitivity of ELISA compared to HPLC for detecting CBZ in tomatoes. Maftouh et al. (2020) have already reported a better sensitivity of the ELISA system than HPLC for pesticide analysis. In many countries where CBZ is banned, the CBZ MRL has been defined as the limit of detection (LOD) using typical analytical methods (Elshafey et al. 2022). An interesting fact is that the high sensitivity of ELISA could help to detect traces of the banned fungicide CBZ, but also the presence of benomyl was reported in the food samples.

Conclusion

A polyclonal antibody in-house ELISA for detecting CBZ in tomatoes using a one-step extraction method was developed. A comparison of the ELISA and HPLC results showed that the agreement between the two techniques was perfect, and the sensitivity of the ELISA was higher. The developed ELISA is handy for the rapid analysis of many samples. This technology can also reduce the MRL of banned pesticides such as carbendazim. In the long term, we aimed to validate the developed ELISA system for determining CBZ in other environmental matrices.

Conflicts of interest

The authors reported no potential conflict of interest.

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