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Research Article

IMMUNODETECTION OF FENTHION IN FOOD SAMPLES USING AVIAN ANTIBODIES

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ABSTRACT

A simple competitive immunoassay was developed for the detection of fenthion in food samples. We employed the avian antibodies (IgY) that recognised fenthion as a capture reagent and fenthion-alkaline phosphatise conjugate as an enzyme label. The assay depended on the competitive binding between the antibody and fenthion extracted from spiked food samples for binding sites with immobilised fenthion-OVA conjugate. The concentration of fenthion in the food samples was quantified by the ability of the pesticide present in food samples to inhibit the binding of the enzyme conjugate to the antibody and subsequently the colour formation in the assay. The assay was specific to fenthion with a limit of detection of 100fg mL^{-1}. Mean analytical recovery of fenthion in different food samples was 13–88%.The precision of the assay was satisfactory. The assay compared favourably with gas chromatography (GC) in its ability to accurately measure fenthion in the food samples.

INTRODUCTION

Pesticides are used globally for enhancing crop yields. However, their excessive use/misuse, especially in the developing countries, results in widespread food and environmental contamination. Fenthion (O,O-dimethyl O-[3-methyl-4-(methylthio)phenyl]phosphorothioate) is a widely used systemic organophosphorus insecticide,avicide, and acaricide (Pesticide Manual A World Compendium, 1997). Fenthion is a contact and stomach insecticide used against many sucking, biting pests. It is particularly effective against fruit flies, leaf hoppers, cereal bugs, stem borers, mosquitoes, animal parasites, mites, aphids, codling moths, and weaver birds. It has been widely used in sugar cane, rice, field corn, beets, pome and stone fruit, citrus fruits, pistachio, cotton, olives, coffee, cocoa, vegetables, and vines[2].Based on its high toxicity on birds, fenthion has been used to control weaver birds and other pest-birds in many parts of the world. Fenthion is also used in cattle, swine, and dogs to control lice, fleas, ticks, flies, and other external parasites (EXTOXNET, 2003, USEPA, 2001, APVMA, 2005). Amid concerns of harmful effects on environment, especially birds, Food and Drug Administration no longer approves uses of fenthion.

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However, fenthion has been extensively used in Florida to control adult mosquitoes. After preliminary risk assessments on human health and environment in 1998 and its revision in 1999, USEPA issued an Interim Reregistration Eligibility Decision (IRED) for fenthion in January 2001. The EPA has classified fenthion as Restricted Use Pesticide (RUP), and warrants special handling because of its toxicity (ASTDR, 2005). Due to its relatively low toxicity towards humans and mammals, fenthion is listed as moderately toxic compound in U.S. Environmental Protection Agency and World Health Organization toxicity class (EXTOXNET, 2003, HSDB, 2003) .Some common trade names for fenthion are Avigel, Avigrease, Entex, Baytex, Baycid, Dalf, DMPT, Mercaptophos, Prentox, Fenthion 4E, Queletox, and Lebaycid (EXTOXNET, 2003).Fenthion is available in dust, emulsifiable concentrate, granular, liquid concentrate, spray concentrate, ULV, and wettable powder formulations. Like most other organophosphates, its mode of action is *via* cholinesterase inhibition. Fenthion exposure to general population is quite limited based on its bioavailability. Common form of fenthion exposure is occupation related, and occurs through dermal contact or inhalation of dust and spray (ASTDR, 2005). Another likely means of contamination is through ingestion of food, especially, if food has been applied quite recently with fenthion. So far, ingestion is the most likely severe poisoning case on humans and animals (HSDB,

2003). To avoid this, crops applied with fenthion should be allowed enough degradation time before harvesting. Normally, 2 - 4 weeks time is enough depending upon the type of crop. Acute poisoning of fenthion results in miosis (pinpoint pupils), headache, nausea/vomiting, dizziness, muscle weakness, drowsiness, lethargy, agitation, or anxiety. If the poisoning is moderate or severe, it results in chest tightness, breathing difficulty, hypertension, abdominal pain, diarrhea, heavy salivation, profuse sweating, or fasciculation. In extremely severe cases, such as suicide attempts, the victim may experience coma, respiratory arrest, seizures, loss of reflexes, and flaccid paralysis (USEPA, 2001, ASTDR, 2005). Photodegradation and biodegradation are common mechanisms of fenthion degradation in the environment. In the atmosphere, vapor phase fenthion reacts rapidly with photochemically produced hydroxyl radicals, and its half-life is about 5 hours. In soil and water, photodegradation is again predominant mechanism if there is enough presence of sunlight. Under normal aquatic environment, half-life of fenthion in water is 2.9 to 21.1 days (HSDB, 2003).

It may be photodynamically, chemically or biologically degraded. The degradation mechanisms can be hydrolysis, oxidation, and/or alkylation-dealkylation, which are dependent on the presence of light, temperature, alkali, or enzymatic activity (Wang et al., 2003). In soil, fenthion degradation ranges from 4 to 6 weeks and it occurs through photodegradation as well as anaerobic or non-photolytic organisms. However, soil particles strongly adsorb fenthion that makes it less susceptible to percolate with water through the soil[5].Due to its extensive application to crops and cattle, residues of fenthion have been detected in food and the environment (Tsatsakis et al 2003).It is also moderately toxic to mammals (LD_{50} values ranging from 88 to 298 $mgkg^{-1}$) and highly toxic to birds ($LD_{50}$from less than 4 to 26 $mgkg^{-1}$), fish (LD_{50} 830 $\mu g\ L^{-1}$)and aquatic invertebrates (LD_{50} 0.84 $\mu g\ L^{-1}$). Several methods have been described for the determination of fenthion such as high performance liquid chromatography (Cabras et al 1988), flow injection (Hernandez et al 1988),colorimetric and bioassay techniques(Devi et al 1986), cholinesterase-based biosensors (Lee et al 2002) and gas chromatography (Arrebola et al 2003) Using these methods, pretreatment of the sample such as oxidation,hydrolysis or coupling of fenthion is needed before analysis. For this reason, more specific, sensitive,rapid and economical analytical methods for the determination of fenthion residues are needed. Immunoassay (IA) technology is such an analysis system with simple, rapid and cost-effective characteristics and widely used in pesticides detection. In this paper we discuss the colorimetric detection of fenthionusing avian antibodies.

MATERIALS AND METHODS

Materials

Pesticide standards, Bovineserum albumin (BSA), ovalbumin (OVA), Alkaline phosphatase, completeand incomplete Freund's adjuvant, Tween 20, used for in this study were purchased from Sigma-Aldrich chemical company. ELISA plates were from NUNC. *p*-NPP, ethanoldiamine, were from SISCO, India. All other chemicals and solvents used in this study were of analytical grade purchased from standard chemical companies such as E.Merck, HiMedia. 22 week old poultry was purchased from Manikya poultry farm, Mysuru.

Hapten synthesis

Synthesis of hapten and production of anti-fenthion IgY antibodies methyl parathion, a structurally related compound was taken according to Chauhan et al (2006). Brifely O,O-dimethyl chloridothiophosphate was added to 4-aminophenol dissolved in acetone followed by anhydrous potassium carbonate and catalyst, 4-N,N-dimethylaminopyridine (DMAP). The mixture was refluxed for 30 min. The reaction was monitored by thin-layer chromatography by the method of Pasha et al.(1976)and the product formation was confirmed. 4-({4- [Dimethoxyphosphorothioyl)oxy]phenyl}amino)-4-oxobutanoic acid (II) O-(4-aminophenyl)-O,O-dimethyl thiophosphate was taken in acetonitrile. Succinic anhydride was added to the solution followed by DMAP. The mixture was heated to 60–80ºC with stirring for 2 h. Water was added and the mixture was extracted with dichloromethane (3 times), the organic layer was separated, washed with brine and dried over anhydrous magnesium sulphate. The solvent was evaporated to dryness to obtain a residue. Residue was analyzed by TLC and product formation was confirmed.

Preparation of active ester

O-[4-({4-[2,5-dioxopyrrolidin-1-yloxy]-4-oxobutanoyl} amino) phenyl] O,O-dimethyl- thiophosphate (active ester of the hapten) (III) was prepared according to Chauhan et al (2006) 4-({4-[Dimethoxyphosphorothioyloxy]phenyl}amino)- 4-oxobutanoic acid (260 mg) was dissolved in dry dichloromethane, N-hydroxysuccinimide was added to the solution and the mixture was cooled to 0ºC and stirred on a magnetic stirrer. N,N -dicyclohexylcarbodiimide (DCC) and DMAP were added and stirring was continued overnight. The dicyclohexylurea was removed by filtration and the solvent evaporated to dryness to obtain the active ester. This was stored desiccated below 0 ºC.

Conjugation of the active ester of hapten to proteins

The hapten–protein conjugate was prepared as follows: Bovine serum albumin (BSA) was dissolved in phosphate buffer pH 9.1 and the solution was cooled to 0ºC. O-[4-({4-[2,5-dioxopyrrolidin-1- yl)oxy]-4-oxobutanoyl}amino)phenyl] O,O-dimethyl thiophosphate dissolved in dimethylformamide (DMF) was added to the solution slowly with swirling. The mixture was stored at ca. 8ºC overnight and dialyzed using 50 mM phosphate buffered saline with three changes at 6h intervals. Similar conjugates were prepared with OVA, ALP and HRP.

Immunization of poultry

White Leghorn birds (layers) were immunized intra-muscularly using hapten–protein conjugate. In the first injection, immunogen-BSA conjugate was taken in Freund's complete adjuvant (FCA) and immunized in the breast muscle. Then periodic immunization (booster injections) was given in Freund's incomplete adjuvant (FICA) with immunogen-BSA conjugate at time intervals 15 days up to 12 weeks. Eggs were collected daily for isolation of antibodies.

Isolation of antibodies

Eggs were collected daily and antibodies were isolated from egg yolk. Yolk was separated from egg white and suspended in PBS.

The egg yolk was broken, made in to uniform suspension, homogenised well in magnetic stirrer for 10 min and was further kept for another 30 min with the addition of chloroform at ambient temperature. The mixture was centrifuged at 10,000 g, at 4ºC for 10 min. The supernatant was decanted and PEG 6000 was added to the supernatant at 14% (W/V) level. This was again mixed well for 30 min at ambient temperature on a magnetic stirrer. Then it was centrifuged at 10,000 g, at 4ºC for 10 min. The supernatant was decanted and the precipitate (IgY) was dissolved in required quantity of PBS and stored at 20ºC.

Optimisation of fenthion detection by ELISA

The protein concentration of IgY was determined by the Bradford method [16] The antibody concentration was determined by ELISA. Antibody (100 µL) isolated from yolk of different weeks was coated on to microwell plates and immobilised at 4ºC overnight. The plates were washed with 0.1% Tween 20 in phosphate buffered saline (PBS: 137 mMNaCl, 3 mMKCl), and the wells were blocked with 1% gelatin (100 µL) by incubation at 37ºC for 1 h. The plates were then washed with 0.1% Tween 20 in phosphate buffered saline. Hapten- ALP conjugate (100 µL) was added to the wells of the plates and allowed to incubate in the microwells at 37ºC for 1 h.

After washing with PBS containing 0.1% Tween 20, pNPP in diethanolamine buffer (pH 9.8), microwell substrate (150 µL) was used for colour development. The reaction was stopped by the addition of 3M NaOH (50 µL). The absorbance of each well was measured in an ELISA reader at 405 nm. The antibody showing highest titre values were used for further studies. The optimum antibody concentration for coating onto the microwell plates and the best working concentration of the enzyme conjugate were determined by checkerboard titration. IgY antibody was diluted with carbonate buffer (Na_2CO_3, $NaHCO_3$ pH 9.6) at concentrations from 2 mg to 1 pg (Bradford protein) and coated onto microwell plates (100 µL) by incubation at 4ºC overnight.

The plates were washed with 0.1% Tween 20 in phosphate buffered saline (PBS: 137 mMNaCl, 3 mMKCl, and 10 mM sodium phosphate, pH 7.4), and the wells were blocked with 1% gelatin by incubation at 37ºC for 1 h. Hapten-ALP conjugate was serially diluted in carbonate buffer at concentrations from 2 mg to 2 pg levels (Bradford protein) and added to the wells of the plates (100 µL) and allowed to incubate in the microwells at 37ºC for 1 h. The remaining procedure is as described above. Concentrations of antibody and enzyme conjugate that showed highest titre values were used for further testing.

Standard curve

All procedures were carried out at 37ºC. Fenthion was premixed with hapten-ALP conjugate and aliquots (144 mg protein mL^{-1}) of the mixture were added to a microwell that had been previously coated with 1: 50,000 dilution of IgY antibody and blocked with 1% gelatin. After 1 h incubation, the plates were washed and the amount of bound hapten-ALP conjugate was quantified using pNPP in diethanolamine buffer (pH 9.8) microwell substrate as described above.

Analysis of food samples

Spiking and Extraction of FenthionFrom Food Samples

Different food commodities, cabbage, brinjal, carrot, beet root, capsicum, tomato, lemon, grapes, and rice were spiked by adding 100ng g^{-1} of fenthion uniformly, mixed well and allowed to stabilize for 24 h at room temperature (25–28ºC). Then all the food materials were extracted with phosphate buffer saline. The fractions in the buffer were pooled (50 mM, pH 7.5) for immunoassay.

Determination of Fenthion in Food Samples by ELISA

Hapten-OVA conjugate (100 µL) (Capture conjugate) was added to the wells of the plates and immobilized at 4ºC overnight. The plates were washed with 0.05% Tween 20 in phosphate buffered saline (PBS-T) (PBS: 137 mMNaCl, 3 mMKCl), and the wells were blocked with 0.2% gelatin (100 µL $well^{-1}$) by incubation at 37ºC for 1 hr. The plates were then washed with PBS-T. The extracts of different food samples spiked with fenthion (100 µL $well^{-1}$) was added to the wells of the plates and allowed to compete with the primary chicken antibody (IgY) (100 µL $well^{-1}$) isolated from egg yolk and allowed to incubate in the microwells at 37ºC for 1 hr. The plates were washed with PBS-T. Rabbit anti-chicken IgG conjugated to ALP (secondary antibody) was added on to the wells (100 µL $well^{-1}$) and allowed to incubate at 37ºC for 1 hr. The amount of antibody conjugated to antigen was determined by using chromogenic substrate p-NPP (1 mg mL^{-1}) in diethanolamine buffer; 150 µL well-^{1} was used for colour development. The reaction was stopped by the addition of 3 M NaOH (50 µL $well^{-1}$). The absorbance of each well was measured in an ELISA reader at 405 nm. Blanks of food commodities prepared without spiking with fenthion were taken as control for matrix effect.

Specificity of the antibody

Few organophosphorus pesticides such as quinolphos, chlorpyrophos, methyl parathion, Fenthion were tested. The ELISA procedure was same as used above. Here instead of fenthion different organophosphorus pesticides were used in competition with primary antibody. Cross reactivity was calculated as the reduction in activity compared to the control (fenthion) and expressed as % relative activity. Pesticide stocks were prepared by dissolving standards at 1 $mgmL^{-1}$ in phosphate buffer saline. Assay was carried out by indirect ELISA as described above

GC analysis

Fenthionstock solution (1mg mL^{-1}) in acetone was diluted to give 100pg μL^{-1} and injected to Shimadzu 2010 Gas chromatographic instrument with the following conditionsInjector:220°C, FPD detector 250°C,column maintained as follows : 80°C with 10 min. hold time, 20°C min^{-1} increase up to 200°C, 6 min. hold time, 20°C min-1 increase to 250°C with 10 min hold time and nitrogen gas at 1 $mLmin^{-1}$ flow rate was used as carrier gas.

Analysis of spiked food samples by GC

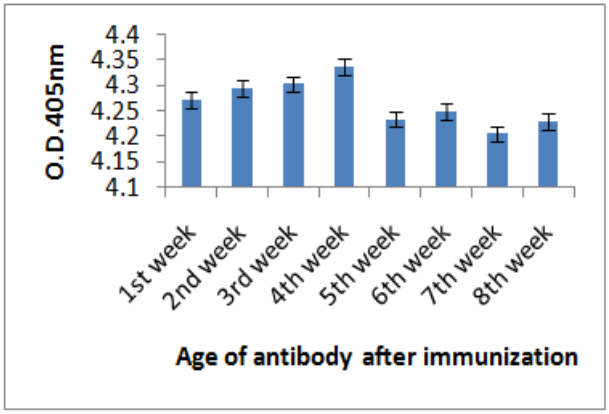
Different food commodities, cabbage, brinjal, carrot, beet root, capsicum, tomato, lemon, grapes, and rice were spiked by adding 100ng g^{-1} of fenthion uniformly, mixed well and

allowed to stabilize for 24 h at room temperature (25–28ºC). Then all the food materials were extracted with hexane. Solvent extract was passed through florisil column and collected over a bed of anhydrous sodium sulphate. The collected solvent extract was evaporated to dryness and resuspended in known volume of acetone and used for GC. Other GC conditions used were same as above. The concentration of fenthion recovery was calculated using the area obtained in the chromatogram.

RESULTS AND DISCUSSION

This study describes a format for an enzyme immunoassay using avian antibodies that quantifies fenthion in different food matrices. Microwell plates were coated first with avian antibody that was raised against hapten-BSA conjugate. Hapten-active ester conjugated with ALP, the form recognised by the antibody was used in the assay. Fenthion solution was mixed with the pesticide-ALP conjugate, and the mixture was incubated with the immobilised antibody in the microwell. During this incubation, the fenthion complexes compete with hapten ALP conjugate for binding sites of the immobilised antibody. After removal of unbound reagents, the amount of enzyme conjugate bound to the antibody was determined using a chromogenic substrate.

The concentration of fenthion in a sample was quantified by the ability of its complex to inhibit the binding of hapten-ALP conjugate to the antibody, and colour development was inversely proportional to the concentration of fenthion in the original sample. In Indirect ELISA, pesticide-OVA conjugate was immobilized on to the ELISA wells. Fenthion solution was mixed with the primary antibody and the mixture was incubated with the immobilised antigen in the microwell. After the reaction rabbit anti chicken secondary antibody conjugated with ALP was added to recognize the attached primary antibodies. The amount of enzyme conjugate bound to the antibody was determined using a chromogenic substrate. The concentration of fenthion in a sample was quantified by the ability of its complex to inhibit the binding of primary antibody to the hapten-ALP conjugate, and colour development was inversely proportional to the concentration of fenthion in the original sample.



Notes: The values are mean ± SD of three independent experiments (n=3).

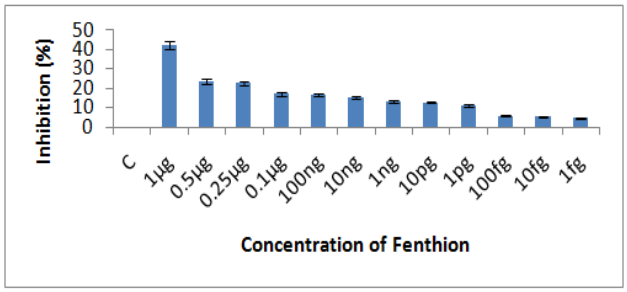
Fig 1 : Antibody titre

Choice of antibody and enzyme conjugate concentrations

The protein analysis of IgY preparations indicated that the antibody of the 4th week had the highest protein concentration. The antibody titre determination in the microwell plates indicated that the antibody of 4th-weekegg yolks had the highest antibody titre (Figure 1). Enzyme-labelled conjugate of pesticide was prepared by reacting active ester of the pesticide hapten with the lysine-amino groups of the BSA, Ovalbumin (OVA) and alkaline phosphatise (ALP) enzymes. The conjugation reaction did not affect the enzyme activity or the immuno reactivity of the conjugate with the immobilised avian antibody. Optimal concentrations of antibody required for coating and the best working concentration of the enzyme conjugate were determined by performing assays using varying concentrations of the conjugate and immobilised antibody. The checkerboard analysis indicated that antigen dilution of 1 : 10^3 (1 mg protein) and antibody dilution of 1 : 10^5 (144 pg protein) gave the optimum readings (data not shown). These concentrations were chosen for further work.

Optimisation of assay condition (Calibration curves and sensitivity)

Carbonate buffer was chosen as the buffer in the present work unless otherwise stated. Calibration curve was generated using fenthion at concentrations from 0.0 to 1µg mL^{-1} , prepared in PBS (Figure 2). The sensitivity of the assay was determined by identifying the limit of detection, defined as the lowest measurable concentration of fenthion that could be distinguishable from zero concentration. On the basis of eight replicate measurements, the limit of detection was 100fgmL^{-1} . The IC_{50} value of this avian antibody was 1.184µgmL^{-1}.



Notes: The concentrations of the pesticide standard quoted on the X-axis werediluted using carbonate buffer. Data were mean of eight independent experiments (n=8).

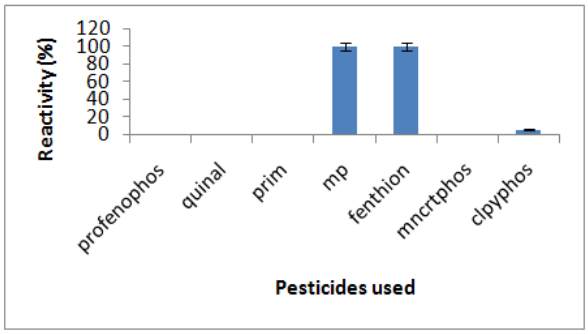
Fig. 2. Immunoassay with different concentrations of fenthion

pesticide specificity

Many organophosphorus pesticides have been used extensively and residues have been observed in the environment. Interaction of the avian antibody was tested as it was necessary to test the ability of organophosphorus pesticides to cross react in the present competitive immunoassay for fenthion. Fig 3 shows the cross reactivity of few individual organophosphorus pesticides added at 100ng mL^{-1} level in the assay. These data were obtained are based upon triplicate determinations. The presence of any of the organophosphorus pesticides except methyl parathion did not interfere in the assay.

Precision and accuracy

The intra- and inter-assay precisions were determined at different fenthion concentrations (0.1µg, 500ng, 250ng, 100ng, 10ng and 1ng).



Notes: Few pesticides were tested for cross-reactivity to the anti-fenthion antibody. Datawere based upon the mean of triplicate determinations. Competitive immunoassays wereperformed as described in the experimental section using analytical grade standard pesticides.

Fig. 3. Cross-reactivity/specificity of fenthion immunoassay

The intra-assay precision was assessed by analysing eight replicates of each sample in a single run, and the inter-assay precision was assessed by analysing the same sample, as duplicates, in four separate runs. The assay gave satisfactory results over all the tested concentration levels; the coefficients of variations were 0.21–0.35 and 0.11–0.43 for intra- and inter-assay precision, respectively (Table 1).

and an absence of endogenous interfering substances in food samples. All the runs were made against respective blanks. The recovery was compared to the GC analysis. (Table2). The results were *in par* with the recovery obtained by immunoassay. Owing to the extensive use of insecticides, there is mountingconcern over biological and environmental contamination. Among the insecticides, fenthion [O,O-dimethyl O-[3-methyl-4-(methylthio)phenyl] phosphorothioate] is anorganophosphorus insecticide used worldwide for the control of many sucking and biting pests(TomLin,. 1997) and it has been shown to cause severe lethal poisoning and be accumulated *via* contact with skin, inhalation, and ingestion.

Due to the high specificity of antibodies immunoassay has enabled one to detect a variety of analytes at very low levels. Among the enzymes, ALP and HRP are used extensively as chromogenic tags for detection, Glucose-6-phosphate dehydrogenase (G6PDH) has also been used as a label in homogeneous EMIT type assays for the detection of fenthion (Kim et al., 2007). Usually rabbit antibodies have been isolated for fenthion detection (Kim et al., 2003). To the best of our knowledge this is the first report on the production of IgY antibodies to detect fenthion. The concentration (ED_{50}) of fenthion required toachieve a 50% reduction in the maximum

Table 1. Precision of the immunoassay for fenthion

Concentration of Fenthion (µg/ml)	Intraassay n=8 SD (µg/ ml)	CV[a]	Inter assay n=8 SD (µg/ ml)	CV[a]
1µg	0.08	0.35	0.08	0.3
500ng	0.21	0.22	0.22	0.42
250ng	0.28	0.21	0.50	0.39
100ng	0.58	0.29	0.84	0.43
10ng	2.65	0.25	1.20	0.12
1ng	5.44	0.21	3.86	0.11

[a]CV is the coefficient of variation

Table 2. Immunoassay of fenthion spiked to different food samples

Food Sample	Recovery of Fenthion by immunoassay (%)	Recovery of Fenthion by GC (%)
Pomegranate	52.23±0.22	50.53 ±0.02
Tomato	31.23 ±1.84	45.22 ±2.24
Sugarcane	39.95±2.44	67.45 ±0.16
Cucumber	51.90 ±0.82	66.23 ±0.22
Capsicum	35.94 ±0.28	43.25 ±3.20
Rice	77.63 ±0.42	79.65 ±1.50
Grape	79.45 ±3.20	79.88 ±1.70
Corn	86.11 ±1.60	88.22 ±1.50
Banana	40.34 ±0.28	54.24 ±1.74
Eggplant	88.66 ±0.56	82.32 ±1.13
Beetroot	52.38±3.20	48.74 ±0.92
Cabbage	60.43±0.96	58.94 ±1.40
Lemon	77.37 ±0.38	80.12 ±1.91
Radish	80.87 ±1.54	78.56 ±2.14
Carrot	85.47±2.16	78.88 ±2.10
Coffee	13.02 ±2.86	14.86 ±3.60

Notes: Samples were collected as described in experimental sections. Values were a mean of triplicatedeterminations ± SD. Competitive immunoassays were performed as described in the experimentalsection.

Analysis of spiked food samples

Recovery of the assay was assessed by adding known amount (100ng mL^{-1}) of fenthion to fenthion-free food samples; each sample was subsequently analyzed in triplicate for its fenthion content. The mean analytical recovery was calculated as the ratio between the fenthion concentration found and the concentration added, expressed as percentage. As shown in Table 2, a quantitative recovery of 13–88% of the added fenthion was obtained, indicating the accuracy of the method

values ofpercent inhibition which can be related to the relative affinity ofthe given antibody toward fenthion (Kim et al 1990) were 0.809, 1.042, and 1.194 µg/ml using G6PDH labelled hapten. In our studies, the IC_{50} of fenthion towards anti-fenthionIgY was observed to be 1.184µg / mL using ALP labelled hapten. The analyte, fenthion could be detected in spiked food matrices. Rapid duplex ELISA for the simultaneous determination of two of the most widely used organophosphorous insecticides, chlorpyrifos and fenthion in

tangerine juices has been reported (Navarro et al 2013). The optimized duplex ELISA was accomplished within 40min achieving a detection limit of 0.20 ± 0.04 µg/L and0.50 ± 0.06 µg/L, for chlorpyrifos and fenthion, respectively in tangerine juice samples.

Conclusion

We have described the performance of a competitive enzyme immunoassay using IgY antibodies that is useful to rapidly and conveniently determine the amount of fenthion. With the appropriately prepared e nzyme-hapten conjugates and anti-hapten antibodies, theproposed assay method may offer a simple method of fenthiondetection in food samples.

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