Sensitivity of Commercial Enzyme Inhibition Colorimetric Pesticide Residue Rapid Test Kit to a Variety of Pesticides

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Abstract [**Objectives**] To fully understand the quality of commercial enzyme inhibition-colorimetric pesticide residue rapid detection kits, so that they can play a greater role in the detection and supervision of agricultural products. [**Methods**] The sensitivity of 28 kinds of pesticides was determined by using the commercially available enzyme inhibition colorimetric rapid detection kit with Hendu brand. [**Results**] There was a significant difference in the sensitivity of the kit to each pesticide, and the kit was more sensitive to dichlorvos among the 28 pesticides tested. The sensitivity to methyl isosalifos, dimethoate, isocarbophos, fenthion and phorate was poor, and the sensitivity to quinalphos was different between 3.0 and 2.5 mL. [**Conclusions**] The large difference of the sensitivity of the enzyme inhibition-colorimetric rapid detection kit for pesticide residues to different kits is a reason for the false positive and false negative test results of the kit, which needs to be considered by relevant personnel.

Key words Enzyme inhibition-colorimetry, Reagent kit, Pesticide, Inhibition rate, Sensitivity

1 Introduction

Based on the principle that organophosphorus and carbamate pesticides inhibit cholinesterase in animals, enzyme inhibition method for rapid detection of pesticide residues has been widely used in the supervision of pesticide residues because of its simple operation, fast detection speed and low technical requirements for testing personnel[1]. The basic reagent of the method comprises an enzyme, a chromogenic reagent, a substrate and a buffer salt, and the core reagent is the enzyme. Due to economic factors, the application of plant esterase in this method has been widely studied in recent years[2-4]. The sources of enzymes in commercial products based on this method lead to different activities of enzymes[5], different sensitivities of enzymes from different sources to pesticides[6], and the methods of enzyme preparation[2,7], separation and purification[7-8] lead to different purities, enzymes from different sources have different requirements for external conditions such as pH[9] and temperature of the reaction system, whether to add a protective agent to protect the enzyme in the assembly of the kit[10], and the enzyme has a certain selectivity for the substrate[2] and the chromogenic agent[11]. Therefore, the sensitivity of commercial enzyme inhibition-colorimetric rapid detection kits for pesticide residues to pesticides is quite different[12], also leading to false-positive and false-negative test results[13]. The sensitivity of the enzyme in the kit to pesticides is one of the important factors to evaluate the quality of the kit[14], and is also the key to solve the problems in the detection work. Therefore, we improved the test in this study. In order to provide some theoretical support for users, we used the commercial rapid detection kit of enzyme inhibition method to determine the sensitivity of 28 kinds of pesticide residues.

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2 Materials and methods

2.1 Instruments Pesticide residue tester (CL-BIII, 16 channels, Shanghai Fubo Agricultural Science and Technology Co., Ltd., Shanghai Bona New Technology Research Institute); double thermostatic water bath oscillator (SHA-C type, Jiangsu Jieruier Electric Appliance Co., Ltd.); vortex mixer (Model WH-861); electric thermostatic water bath (Model HH. W21-Cr 420, Beijing 328 Scientific Instrument Co., Ltd.); Thermo 200 μL and Rongtai 5 mL pipette.

2.2 Reagents

2.2.1 Kit (Hendu brand, Dongguan); test water: purified water. Before use, the buffer salt was dissolved in 500 mL of purified water; the enzyme and developer were prepared with 3.0 mL of buffer solution, and the substrate was prepared with 3.0 mL of purified water.

2.2.2 Selection of pesticide varieties. We selected 23 kinds of organophosphorus pesticides (OP) including quinalphos, dichlorvos, chlorpyrifos, parathion, phosalone, dipterex, methidathion, omethoate, triazophos, malathion, coumaphos, monocrotophos, profenofos, methamidophos, diazinon, fonofos, methyl parathion,

phoxim, methyl isosalifos, dimethoate, isocarbophos, fenthion, and phorate, and 5 kinds of carbamate pesticides (CP) including thiophosphoryl chloride, carbofuran, thiodicarb, aldicarb sulfone, and alidcarb. The pesticide standard is from the Agricultural Environmental Protection Research and Testing Institute, with a concentration of 1 000 mg/L. Pesticides were diluted into a stock solution of 80 μg/mL, and pipetted as required during the experiment.

2.3 Experimental design Five concentration gradients were set for each pesticide. The preparation method comprises the following steps: taking a proper amount of pesticide standard substance into a disposable beaker, weighing the sample at 2.0 g, so that the mass fraction of the pesticide is 0.05, 0.5, 1.0, 2.0 and 3.0 mg/kg, and repeating for three times at each concentration level.

2.4 Test methods Test of control solution and pesticide: the test method of control solution and pesticide shall be in accordance with the national standard[15]. Since the volume of the sample extract in the national standard[15] and industry standard[16] tests is 2.5 and 3.0 mL, respectively, the sample extraction volumes of 2.5 and 3.0 mL were set in this test.

We took a number of 10 mL disposable test tubes, separately transferred 2.5 and 3.0 mL of buffer solution and sample extract, added 100 μL of enzyme and 100 μL of chromogenic reagent in turn, vortexed and mixed well, and conducted water bath at 37 ℃ for 10 min. Then added 100 μL of substrate and shook it up, put the control solution into the first channel colorimetric cell of the 16-channel CL-BIII pesticide residue rapid tester for detection, and read the change of absorbance within 3 min through the instrument. The treated pesticide samples were sequentially put into the colorimetric pools of other channels of the pesticide residue rapid tester for detection. The suppression rate of the following channel was directly read out by the instrument and recorded. The instrument automatically calculated the inhibition rate according to the change value of the absorbance of the control measured in the first channel within 3 min.

2.5 Processing of experimental data (i) Selected the concentration value between 0 – 100% of the weighted average of the inhibition rate of five pesticides measured three times at each concentration level, and used Excel to linearly fit the scatter plot of the inhibition rate and the common logarithm of the pesticide concentration, so as to obtain the concentration-effect curve equation, the correlation coefficient and the half inhibition concentration IC_{50} of each pesticide to each kit. According to the concentration-effect curve equation $y = kx + b$, the value of the x-axis corresponding to $y = 50\%$ is obtained, and $IC_{50} = 10^x$; the linear range is the range between the maximum concentration value with the inhibition rate of 0 and the concentration value corresponding to the measured maximum inhibition rate; in similar way, we obtained IC_{10}. The national food safety standard[17] stipulates that the maximum limits of omethoate, methamidophos and dipterex in vegetables are 0.02, 0.1 and 0.5 mg/kg, respectively; the maximum IC_{50} values of these three pesticides determined by individual kits were 824[18], 3.84[12] and 1.82×10^4 mg/kg[19], respectively. The corresponding IC_{10} values are 0.610[18], 0.200[12] and 7.35 mg/kg[19], respectively, which are greater than the national limit standards. Therefore, IC_{10} was set as the detection limit of the kit to compare the sensitivity of different kits to different pesticides; the lower the IC_{10}, the more sensitive the kit was to the pesticide. When $IC_{10} < 10^{-3}$ mg/kg, the kit was extremely sensitive to pesticides; when 10^{-3} mg/kg $< IC_{10} < 10^{-1}$ mg/kg, the kit was more sensitive to pesticides; when 10^{-1} mg/kg $< IC_{10} < 1.0$ mg/kg, the kit was less sensitive to pesticides; when $IC_{10} > 1.0$ mg/kg, the kit was not sensitive to pesticides.

(ii) According to the results of (1), the influence of sample extraction volume of 2.5 and 3.0 mL on the determination results and the sensitivity of the kit to different organophosphorus pesticides were compared.

3 Results and analysis

No matter 3.0 mL or 2.5 mL of sample extract was used, there was no linear relationship between the inhibition rate and the pesticide concentration when fonofos, methyl parathion and phoxim were determined by Hendu kit; when methyl isosalifos, dimethoate, isocarbophos, fenthion and phorate were determined, the inhibition rates were 0 at the five concentrations set, indicating that the enzyme in the kit had very low sensitivity to these six pesticides. The other 20 pesticides were ranked according to their IC_{10}, as shown in Table 1 and Table 2.

3.1 Sensitivity analysis of Hendu kit to 28 kinds of pesticides From Table 1, it can be known that when 3.0 mL of the extract was taken, the sensitivity of Hendu kit to 28 kinds of pesticides was quinalphos, dichlorvos, thiophosphoryl chloride, chlorpyrifos, parathion, carbofuran, phosalone, dipterex, methomyl, methidathion, omethoate, triazophos, aldicarb sulfone, malathion, coumaphos, alidcarb, monocrotophos, profenofos, methamidophos, diazinon, fonofos, methyl parathion, phoxim, methyl isosalifos, dimethoate, isocarbophos, fenthion, and phorate from high to low. The kit was very sensitive to quinalphos and dichlorvos, and the IC_{10} was below 10^{-3} mg/kg, accounting for 7.14% of the total tested pesticides; the kit was more sensitive to 10 pesticides: thiophosphoryl chloride, chlorpyrifos, parathion, carbofuran, phosalone, dipterex, methomyl, methidathion, omethoate, triazophos was, and its IC_{10} was between 10^{-3} and 10^{-1} mg/kg, accounting for 35.7% of the total tested pesticides; the kit was not sensitive to eight pesticides (aldicarb sulfone, malathion, coumaphos, alidcarb, monocrotophos, profenofos, methamidophos, diazinon), and its IC_{10} was between 10^{-1} and 1.0 mg/kg, accounting for 28.6% of the total tested pesticides; the kit was not sensitive to methyl isosalifos, dimethoate, isocarbophos, fenthion and phorate, and its IC_{10} was 1.0 mg/kg, accounting for 17.9% of the total tested pesticides.

Table 1 Sensitivity of the kit to 23 kinds of pesticides when 3.0 mL of the extract was taken

Pesticide	Inhibition rate equation	Linear range // mg/kg	R^2	IC_{50} // mg/kg	IC_{10} // mg/kg
Quinalphos	$y=0.633\ 2x+99.605$	0.05 – 1.0	0.461 1	4.57×10^{-78}	2.450×10^{-90}
Dichlorvos	$y=1.8x+100.54$	0.05 – 0.5	1.000 0	8.51×10^{-29}	5.010×10^{-51}
Thiophosphoryl chloride	$y=29.244x+81.214$	0.05 – 3.0	0.976 3	0.085 62	3.671×10^{-3}
Chlorpyrifos	$y=49.2x+114.81$	0.05 – 0.5	1.000 0	0.048 16	7.408×10^{-3}
Parathion	$y=62.887x+107.75$	0.05 – 1.0	0.956 4	0.120 7	0.027 9
Carbofuran	$y=39.927x+65.219$	0.05 – 3.0	0.998 2	0.415 7	0.041 4
Phosalone	$y=71.043x+102.47$	0.05 – 1.0	0.996 4	0.182 6	0.0499
Dipterex	$y=62.155x+89.991$	0.05 – 2.0	0.972 6	0.227 3	0.052 0
Methomyl	$y=39.411x+56.551$	0.05 – 3.0	0.962 7	0.682 0	0.065 9
Methidathion	$y=52.122x+69.069$	0.05 – 3.0	0.981 7	0.430 6	0.073 6
Omethoate	$y=55.285x+67.91$	0.05 – 3.0	0.926 1	0.474 2	0.089 7
Triazophos	$y=42.136x+52.263$	0.05 – 3.0	0.858 0	0.883 7	0.099 3
Aldicarb sulfone	$y=31.137x+40.817$	0.05 – 3.0	0.959 0	1.972 0	0.102 0
Malathion	$y=38.01x+46.663$	0.05 – 3.0	0.928 4	1.224 1	0.109 0
Coumaphos	$y=41.389x+48.16$	0.05 – 3.0	0.842 6	1.107 8	0.120 0
Alidcarb	$y=26.864x+34.468$	0.05 – 3.0	0.829 2	3.789 2	0.123 0
Monocrotophos	$y=38.84x+43.313$	0.05 – 3.0	0.878 9	1.486 6	0.139 0
Profenofos	$y=47.863x+50.087$	0.05 – 3.0	0.796 3	1.004 2	0.145 0
Methamidophos	$y=31.14x+31.791$	0.05 – 3.0	0.710 4	3.843 3	0.200 0
Diazinon	$y=9.319\ 5x+10.596$	0.05 – 3.0	0.716 5	$1.690\ 8\times10^{4}$	0.863 0

Table 2 Sensitivity of the kit to 20 kinds of pesticides when 2.5 mL of the extract was taken

Pesticide	Inhibition rate equation	Linear range // mg/kg	R^2	IC_{50} // mg/kg	IC_{10} // mg/kg
Dichlorvos	$y=7.5x+102.26$	0.05 – 0.5	1.000 0	1.076×10^{-7}	4.997×10^{-13}
Thiophosphoryl chloride	$y=30.754x+82.892$	0.05 – 3.0	0.976 5	0.085 21	$4.263\ 8\times10^{-3}$
Chlorpyrifos	$y=60.8x+118.3$	0.05 – 0.5	1.000 0	0.075 27	0.016 55
Dipterex	$y=67.371x+106.51$	0.05 – 1.0	0.972 8	6.899 0	0.030 00
Parathion	$y=66.813x+108.04$	0.05 – 1.0	0.958 3	0.135 3	0.034 09
Carbofuran	$y=41.441x+64.077$	0.05 – 3.0	0.999 4	0.457 4	0.049 56
Phosalone	$y=62.647x+90.401$	0.05 – 2.0	0.969 4	0.226 5	0.052 10
Diazinon	$y=6.4453x+17.422$	0.05 – 3.0	0.483 5	$1.133\ 7\times10^{5}$	0.070 55
Methomyl	$y=42.322x+55.528$	0.05 – 3.0	0.989 0	0.740 3	0.083 98
Methidathion	$y=50.364x+62.679$	0.05 – 3.0	0.954 8	1.785 3	0.089 95
Malathion	$y=34.827x+44.139$	0.05 – 3.0	0.847 4	1.473 3	0.104 70
Omethoate	$y=50.256x+54.061$	0.05 – 3.0	0.792 5	0.830 2	0.132 80
Triazophos	$y=39.461x+43.642$	0.05 – 3.0	0.835 2	1.449 1	0.140 40
Aldicarb sulfone	$y=29.259x+34.817$	0.05 – 3.0	0.852 6	3.302 9	0.141 80
Alidcarb	$y=28.286x+33.391$	0.05 – 3.0	0.827 3	3.864 6	0.149 00
Coumaphos	$y=41.14x+42.359$	0.05 – 3.0	0.723 0	1.533 6	0.163 50
Profenofos	$y=44.569x+44.584$	0.05 – 3.0	0.707 3	1.323 1	0.167 50
Monocrotophos	$y=32.736x+31.451$	0.05 – 3.0	0.648 6	3.686 4	0.221 20
Methamidophos	$y=67.466x+16.803$	0.50 – 3.0	0.932 7	3.105 3	0.792 90

From Table 2, it can be known that when 2.5 mL of the extract was taken, the sensitivity of Hendu kit to 28 kinds of pesticides was dichlorvos, thiophosphoryl chloride, chlorpyrifos, dipterex, parathion, carbofuran, phosalone, diazinon, methomyl, methidathion, malathion, omethoate, triazophos, aldicarb sulfone, alidcarb, coumaphos, profenofos, monocrotophos, methamidophos, quinalphos, fonofos, methyl parathion, phoxim, methyl isosalifos, dimethoate, isocarbophos, fenthion, and phorate from high to low. The kit was highly sensitive to dichlorvos, and its IC_{10} was below 10^{-3} mg/kg, accounting for 3.57% of the total tested pesticides; the test kit was sensitive to 9 kinds of pesticides (thiophosphoryl chloride, chlorpyrifos, dipterex, parathion, carbofuran, phosalone, diazinon, methomyl, and methidathion), and its IC_{10} of was between 10^{-3} and 10^{-1} mg/kg, accounting for 32.1% of the total tested pesticides; the kit was not sensitive to 9 kinds of pesticides (malathion, omethoate, triazophos, aldicarb sulfone, alidcarb, coumaphos, profenofos, monocrotophos, and methamidophos), and its IC_{10} was in the range of 10^{-1} – 1.0 mg/kg, accounting for 32.1% of the total tested pesticides; the kit was not sensitive to five pesticides (methyl isosalifos, dimethoate, iso-

carbophos, fenthion, and phorate), and its $IC_{10} > 1.0$ mg/kg, accounting for 17.9% of the total tested pesticides.

It can be seen from the data in Table 1 and Table 2 that for quinalphos, there was a big difference between 3.0 and 2.5 mL of the sample extract. When 3.0 mL was used, there was a certain linear relationship between the inhibition rate and the logarithm of the concentration of quinalphos, and the linear coefficient is 0.461 1; when 2.5 mL was used, the measured data is messy. The relationship between the inhibition rate and the logarithm of quinalphos concentration is irregular, and the reason needs to be further studied.

4 Conclusion and discussion

The results showed that the sensitivity of Hendu kit to methyl isosalifos, dimethoate, isocarbophos, fenthion and phorate was extremely low among the 28 kinds of tested pesticides from the perspective of IC_{10}. No matter whether 3.0 or 2.5 mL of the sample extract was used, the inhibition rates of these pesticides were all 0 at the five pesticide concentration levels.

In the actual sample detection, when the sample contains any one or more of the above five pesticides, and its content is above the limit stipulated in the national food safety standard *Maximum Residue Limits of Pesticides in Food*[17], but below 3.0 mg/kg, the use of Hendu kit for detection will lead to false negative results. In addition, when the content of dichlorvos in the sample was between 1.076×10^{-7} mg/kg and the limit specified in the national food safety standard *Maximum Residue Limit of Pesticides in Food*[17], the use of the kit will produce false positive results.

In summary, the significant difference in the sensitivity of the kit to various pesticides is one of the important reasons for the false positive and false negative of the test sample. In addition, when the sample contains diazinon, no matter whether 3.0 mL or 2.5 mL of sample extract was used, the content of diazinon in the sample should be more than 16 000 mg/kg according to the judgment threshold of 50%. This result is not only contrary to the requirements of the maximum residue limit of pesticides in food[17], but also deviates from the original intention of sample detection. Therefore, it is suggested that the relevant national standards and industry standards should be revised to adjust their judgment inhibition values so as to reflect the actual situation of the samples more accurately.

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