Investigation of Spectrophotometric Methods for Determination of Organophosphorus Insecticides and its Application to Fruit Samples (Part-II)

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Summary: An indirect spectrophotometric method based on the complexation of copper with insecticides was investigated. The method was checked for its percent recovery of insecticides in various fruits like plums, apricots and grapes. The recovery of the method was found to be 94%. The method was also applied for malathion residue determination in selected fruits. Limit of detection of the method was investigated and was found to be 0.2μg/ml.

Introduction

The use of organophosphorus insecticides for control of insects on fruits and vegetables has increased rapidly during the past few years. However, the use of insecticides on these plant materials poses many problems. These problems occur directly or indirectly from indiscriminate usage. The insecticides are carried in run-off and drainage from soil to water and also finds their way into various food products.

The widespread use of toxic organophosphorus insecticides has created a need for sensitive method for detecting them as spray residue on plant materials, fruits and various types of food stuffs. Different analytical techniques may be used to determine insecticides in water and food products. Among these spectrophotometric method [1-4], polarographic [5-7], GC [8], HPLC [9] and spectrofluorimetric [10] together with various clean-up techniques are the preferred methods for determination of insecticides. In this paper a simple spectrophotometric method for determination of insecticides in fruits sample is presented.

Experimental

Apparatus

Jasco:UVIDEC-1 double beam U.V. visible spectrophotometer, WPA CD 660 digital pH meter were used during this investigation.

Reagents

Samples of malathion and diazinon were obtained commerically and were used without further purification. Purified ethanol and benzene were used for solution preparation and for extraction of insecticides respectively. French chalk was also obtained commerically and was used without further treatment.

Solutions

a) Stock Solution of Cation Cu^{2+})

1000 ppm stock solution of Cu^{2+} was prepared by dissolving an appropriate amount of analytical reagent grade $CuCl_2.2H_2O$ salt in ethanol. This solution was prepared fresh each day.

b) Stock Solutions of Insecticides

1000 ppm stock solutions of malathion and diazinon were prepared by dissolving an appropriate amount of these insecticides in ethanol. 100 ppm stock solutions were prepared from 1000 ppm solution by dilution, using ethanol as a solvent.

Sodium Ethoxide Solution

Sodium ethoxide solution was prepared by dissolving an appropriate amount of sodium metal in ethanol. This solution was stored for further use.

Procedure

Two mls of insecticide solution from 1000 ppm stock solution were taken and to this solution two mls of 1000 ppm metal solution was added. This solution was diluted upto 20 mls with ethanol and two drops of NaOEt solution was added. The solutions were checked for complex formation using U.V./visible spectrophotometer. For wave-

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length optimization studies of the resultant mixture, the solutions were subjected to variable wavelengths studies in the range of 300-600 nm. For determination of suitable pH for complexation, pH of the solution was varied by dropwise addition of NaOEt and measuring the pH by pH meter. The absorbance of these solutions were noted at the optimum wavelength using synthetic blank solution as a reference.

Preparation of a Standard Curve

For preparation of standard curve aliquots of standard solution of malathion of different concentration were taken in separate beakers. Each aliquot was diluted to 10 ml with benzene, and for a blank 10 ml benzene was placed in another beaker. The volume of benzene was reduced by evaporation to 1 ml. This solution was diluted to 10 ml with ethanol followed by the addition of 1 ml of 1000 ppm copper solution. The pH of the solution was adjusted to pH 6 by the addition of NaOEt. The resulting solution was diluted to 20 ml with ethanol. The same procedure was adopted with the blank except the addition of insecticide. The absorbance of the solution was noted at 325 nm. Standard curve was prepared by plotting absorbance against concentration.

Determination of Malathion in Fruits.

To check the recovery of the insecticide by this method, plums were cut into slices. 50 g of the sliced sample was taken to which different concentration of malathion was added and then 10 ml of benzene was added and shaked for 30 minutes. Filtered and washed with 5 ml of benzene. The filtrate was evaporatedon a water bath to 1 ml followed by dilution with ethanol upto 10 ml. The remaining procedure was the same as applied for the standards.

Determination of Malathion in the extract after Treating it with French Chalk

The fruit extracts of benzene were light yellow in color. For removal of this color the extract was stirred with one gram of french chalk until completely wetted and then filtered the solution. Evaporated the filtrate on a water bath to 1 ml followed by dilution with ethanol upto 10 ml. The remaining procedure was the same as applied for standard.

Results and Discussion

As most of the phosphorus containing compounds acts as a good chelating agents due to the nucleophilic behaviour of phosphorus, therefore the complexation ability of organophosphorus insecticides with various metals, for its spectrophotometric determination was investigated. Among the metals tried for complexation, only Cu^{2+} showed promising results.

pH optimization studies were carried out for maximum complexation of Cu^{2+} with malathion and diazinon. Both of these insecticides were found to have maximum complexation at pH 6.

It was observed, that two approaches could be utilized for spectrophotometric determination of insecticides based on its complexation with Cu^{2+}, (i) Direct (ii) indirect approach. In the direct method both the concentration of Cu^{2+} and insecticides were varied and an increase in absorbance was observed. In this case for each sample and standard a separate blank was used to compensate for the absorbance of soluble $Cu(OH)_2$ if any. With the increase in concentration of insecticide, the complexation would also increase and as a result increase in the absorbance was observed. This method was applied for the estimation of the limit of detection of insecticides and the results are given in Tables 1 and 2.

Table-1: Limit of Detection of Cu-Diazinon Complex by Direct Method.

Conc. of Diazinon (μg)	Conc. of Cu Added (μg)	Abs.
0.2	0.2	0.000
1.0	1.0	0.003
2.5	2.5	0.008
5.0	5.0	0.013
10.0	10.0	0.025
20.0	20.0	0.053

Table-2: Limit of Detection of Cu-Malathion Complex by Direct Method.

Conc. of Malathion (μg)	Conc. of Cu Added (μg)	Abs.
0.2	0.2	0.004
1.0	1.0	0.008
2.5	2.5	0.011
5.0	5.0	0.017
10.0	10.0	0.030
20.0	20.0	0.056

In the indirect method, the concentration of Cu^{2+} was kept constant while the concentration of

insecticide was varied. pH of the solution was always adjusted to pH 6 using NaOEt. With the increase of insecticide concentration their is a decrease in the absorbance. This decrease is due to the fact that initially $Cu(OH)_2$ is present which has greater absorbance in the absence of insecticide. When the concentration of insecticide is increased, a decrease in the absorbance is observed due to the fact that some of the Cu^{2+} is complexed with insecticide. The relationship between absorbance and insecticide concentration utilizing this method is given in Table 3 and 4. Insecticide upto the concentration of 0.2 ppm could be detected by this method. This method was also then applied to fruit samples for investigation of percent recovery of insecticide and the results are given in Tables 5,6.

Table-3: Limit of Detection of Cu-Diazinon Complex by Indirect Method.

Conc. of Diazinon (μg)	Abs.
0.0	0.078
0.2	0.075
1.0	0.064
5.0	0.056
10.0	0.054
20.0	0.050

Table-4: Limit of Detection of Cu-Malathion Complex by Indirect Method.

Conc. of Malathion (μg)	Abs.
0.0	0.081
0.2	0.079
0.5	0.073
1.0	0.68
5.0	0.060
10.0	0.055
20.0	0.050

Optimization of Solvent for the Extraction of Insecticides

Various solvents were investigated for the extraction of insecticides from fruits. Among the solvents like water, ethanol, acetone and benzene, the most suitable solvent was found to be benzene. The reasons for selecting benzene are: (1) The insecticides are easily soluble in this solvent; (2) The solvent is low boiling point solvent and during evaporation the loss of insecticides was found to be low; (3) The colour extracted from fruits with this solvent is the minimum. Other solvents like water, ethanol and acetone showed unsatisfactory results because they easily break the cellular structure producing highly coloured extracts. The coloured components of these solutions are not easily removed by common adsorbents. Also the use of adsorbent is not advisable as it would remove some concentration of insecticides apart from decolourizing the extract solution.

Extraction Time

For the estimation of insecticides from fruits the extraction time was investigated and a minimum of 30 minutes extraction time has been employed. The extra time does not affect the extraction efficiency of insecticide except the soft fruits coloring material.

Recovery Test

The recovery of insecticides from fruits were investigated. The results are given in Table 5. Due to the presence of light yellow colour in benzene extracts, these extracts were then treated with french chalk and the concentration of the insecticides was determined. The results are given in Table 6. As can be seen from Table 5, that 94% of the added malathion is recovered in benzene extract. If the extract is treated with french chalk for colour removal from the extract, the recovery is in the range of 73-83%. This indicates that a part from colour removal, some of the inseciticide is also adsorbed on the french chalk and the recovery is dropped as could be seen from Table 6. As little colour is ex-

Table-5: Recovery of Malathion from the Extract. After Evaporation of Benzene

Malathion Added (μg)	% Recovery 1	2	3	X	s
0.025	94.8	94.0	95.0	94.6	0.53
0.50	94.0	92.6	95.0	93.9	1.20
0.75	95.0	94.0	93.6	94.2	0.72
1.00	92.6	93.7	94.0	93.0	1.00

X = Mean value
s = Standard Deviation

Table-6: Recovery of Malathion in the Extract After Treating it with French Chalk

Malathion Added (μg)	X
0.25	73.4
0.50	82.8
0.75	82.5
1.00	78.0

X = Mean value

tracted with benzene which does not interfere with the determination of insecticide by this method, therefore the benzene extract was directly utilized for the determination of malathion residue and the use of french chalk was avoided.

This method was applied for the determination of malathion residue in fruits like plums, apricots and grapes. The malathion residue in plums, apricots were less than 0.2 ppm and in grapes it was zero.

Conclusion

The indirect method based on complexation of insecticides could be applied for insecticides determination in various samples of environmental importance. The use of adsorbent for decolorization is eliminated as the benzene extract is almost colorless and do not interfere at the wavelength of interest. The method is simple, quicker as well as sensitive and does not need expensive instrumentations.

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