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Detection of Methyl Parathion Residues in Vegetables by Using Chromogenic Reagent

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A B S T R A C T

In the present investigation the organophosphorus insecticides *viz.*, chlorpyrifos, dichlorvos, malathion, methyl parathion, monocrotophos, phosphamidon, profenofos, quinalphos and triazophos insecticides were assessed with different chromogenic reagents. Initial screening of the chromogenic reagents to detect organophosphorus insecticides was done through paper strip method. In paper strip method, development of yellow colour was observed for methyl parathion using sodium hydroxide as chromogenic reagent. Phosphamidon and quinalphos were detected by developing brown and white colour using silver nitrate and sodium hydroxide. The darkness of colour on the spot was increased with increased concentrations. The fortification studies were conducted to determine the methyl parathion, phosphamidon and quinalphos through paper strip method. The results reported that using sodium hydroxide yellow colour was appeared in methyl parathion residual extract. Where as in phosphamidon and quinalphos, the residual extract when treated with silver nitrate the colour obtained was not comparable with standards. Validation was done for methyl parathion using standards and residual extracts and the results were comparable with standards as well fortified residual extracts. From the above experimental results, a test kit was developed for methyl parathion residues detection. The developed test kit was used to determine the pesticide residues present in market vegetable samples.

Introduction

Vegetables such as tomato, cabbage, cauliflower, brinjal, bhendi and green leafy vegetables are prone for heavy pest infestation. Hence pesticides are largely used in these vegetables. Sometimes pesticides are also used even after the harvest of the crop. The presence of pesticide residues is a concern for consumers as they pose toxic effects such as interfering with the reproductive systems and foetal development as well as their capacity to cause cancer and asthma (Gilden *et al.*, 2010). The analysis of pesticide residues is an important concern due to their high toxicity and the serious risk that they represent for the human health and environment.

Over the past four decades, extensive researches have produced several sophisticated analytical methods for pesticide residue determination, such as spectrophotometry, gas chromatography, high performance liquid chromatography, gas chromatography coupled with mass spectrometry. However, such methods, although sensitive and accurate, are expensive

and tedious, require complicated pretreatment of the sample and highly qualified technicians to perform them. Hence the development of a simple and rapid analytical method to detect pesticide residue is essential for easy detection.

Materials and Methods

Experimental location

The experiments were conducted in Pesticide Toxicology Laboratory, Department of Agricultural Entomology and Department of Soil Science and Agricultural Chemistry, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore.

Pesticides

Technical grade organophosphorus insecticides which are commonly used for control of pests and diseases in vegetables were used in the present study.

Organophosphorus insecticides used in this study include chlorpyrifos, dichlorvos, malathion, methyl parathion, monocrotophos, phosphamidon, profenofos, quinalphos, triazophos and neonicotenoid insecticide imidacloprid and fungicide copper oxy chloride.

Reagents

All reagents and chemicals used were of analytical reagent grade.

Reagents used in this experiment include ferric chloride, iodine, methyl red, potassium chloride, silver nitrate, sodium carbonate, sodium chloride, sodium hydroxide, sodium thiosulphate and sodium sulphide and were supplied by E-Merck and Sigma chemicals, Mumbai.

Whatman No. 3 filter paper was used in the paper strip method. Glass distilled water was used throughout the experiment.

Sample collection

Fresh vegetable samples were bought from local markets in Coimbatore.

Glasswares

Borosil made glasswares were cleaned with soap solution then rinsed with acetone and distilled water, dried and sterilized in hot air oven at 80°C for three hours before use.

Standard solution preparation

Standard pesticide stock solutions were prepared by dissolving appropriate amount of the analytes in acetone to obtain a concentration of 1000 ppm for the pesticides chlorpyrifos, dichlorvos, malathion, methyl parathion, monocrotophos, phosphamidon, profenofos, quinalphos, triazophos and imidacloprid. Working standard solutions were prepared by diluting the standard stock solutions with acetone.

Screening of chromogenic reagents using paper strips for rapid test kit

Determination of OP insecticides using silver nitrate treated paper strip

Whatman No. 3 filter paper was treated with 1 % silver nitrate solution and allowed to dry at room temperature. Then the filter paper was spotted with 10 ppm standards of chlorpyrifos, dichlorvos, malathion, methyl parathion, monocrotophos, phosphamidon, profenofos, quinalphos and triazophos pesticides by using microlitre syringe.

Later the colour development on the spot was observed.

Determination of OP insecticides using sodium hydroxide treated paper strip

Whatman No.3 filter paper was spotted with 10 ppm standards of chlorpyrifos, dichlorvos, malathion, methyl parathion, monocrotophos, phosphamidon, profenofos, quinalphos and triazophos.

The pesticides spotted filter paper was then treated with 10 % sodium hydroxide solution and then allowed to dry at room temperature. The development of colour on the spot was observed. Lower concentrations were tried for the pesticides which exhibited colour on the spots.

Determination of OP insecticides using silver nitrate and sodium hydroxide treated paper strip

Whatman No. 3 filter paper was treated with 1 % silver nitrate solution and allowed to dry at room temperature. Then it was spotted with 10 ppm standards of chlorpyrifos, dichlorvos, malathion, methyl parathion, monocrotophos, phosphamidon, profenofos, quinalphos and triazophos. Finally the pesticides spotted filter paper was treated with 10 % sodium hydroxide solution.

Later the colour development on the spot was observed. Lower concentrations were tried in pesticides which showed colour spots.

Determination of OP insecticides using methyl red treated paper strip

Whatman No.3 filter paper was treated with 1 % methyl red solution and allowed to dry at room temperature. Then it was spotted with 10 ppm standards of chlorpyrifos, dichlorvos, malathion, methyl parathion, monocrotophos, phosphamidon, profenofos, quinalphos and triazophos. Later the development of colour on the spot was observed.

Determination of OP insecticides using sodium carbonate and methanolic ferric chloride treated paper strip

To detect the organophosphorus insecticides, whatman No.3 filter paper was treated with 20 % sodium carbonate solution and allowed to dry at room temperature. It was spotted with 10 ppm standards of chlorpyrifos, dichlorvos, malathion, methyl parathion, monocrotophos, phosphamidon, profenofos, quinalphos and triazophos by using microlitre syringe. Then the spotted filter paper was treated with methanolic ferric chloride solution and allowed to dry. Then the colour development on the spot was observed.

Extraction of pesticide residues in fortified vegetables for validation

The pesticides which were responded to the chromogenic reagents were taken for validation. The determination of pesticide residues in vegetables was simplified by using acetone extraction. 10 g of vegetable (Cauliflower) was cut into small pieces and it was fortified with different concentrations of pesticide standards and homogenized. 10 ml of acetone was added to the homogenized mixture and shacked well for 2 minutes. Later the mixture was kept undisturbed for 15 minutes. The supernatant solvent was used for pesticide detection.

Determination of selected pesticides using paper strip in fortified vegetable

The selected pesticides methyl parathion, phosphamidon and quinalphos were validated using the following determination procedures.

Determination of methyl parathion in fortified vegetable

Working standards of methyl parathion was prepared at different concentrations of 1, 5

and 10 ppm and fortified on to the 10 g of vegetable. It was then allowed to stand for three hours. Then extract of the sample was taken as described earlier. The standards and fortified sample extracts were spotted on filter paper and treated with sodium hydroxide solution. Later development of the colour on the spot was observed.

Determination of phosphamidon and quinalphos in fortified vegetable

Working standards of phosphamidon and quinalphos were prepared at different concentrations of 2, 4, 6, 8 and 10 ppm and fortified on to the vegetable. It was allowed to stand for three hours. Then the extract of the sample was taken as described earlier. The standards and sample extracts spots were spotted on silver nitrate treated paper strips and treated with sodium hydroxide. The development of the colour on the spot was observed.

Validation of methyl parathion

Methyl parathion standards and residual extract were prepared at different concentrations 1, 5 and 10 ppm along with control. The prepared methyl parathion standard and residual extract were spotted on the filter paper and treated with sodium hydroxide. The development of the colour on the spot of methyl parathion standard and residual extract were observed.

Development of test kit for methyl parathion

A rapid test kit was developed for the detection of the responded organophosphorus pesticides *viz.*, methyl parathion which was responded to colour development through paper strip method. The test kit was a qualitative screen for the detection of methyl parathion based on the colour spot development using paper strip treated with corresponding chromogenic reagents.

Monitoring of methyl parathion in market samples using developed rapid test kit

Vegetables *viz.*, bhendi, brinjal, cabbage and cauliflower were chosen to evaluate the suitability of the developed kit for the analysis of methyl parathion. The selected vegetables were collected from local markets and stores of Coimbatore, Tamil Nadu at weekly intervals to monitor the presence of residues such as methyl parathion. These vegetables were selected because they are highly prone for pest and disease attack and amenable for high pesticide use.

Results and Discussion

Screening of chromogenic reagents using paper strips for rapid test kit

Determination of OP insecticides using silver nitrate treated paper strip

The silver nitrate treated filter paper was spotted with 10 ppm of chlorpyrifos, dichlorvos, malathion, methyl parathion, monocrotophos, phosphamidon, profenofos, quinalphos and triazophos. All the organophosphorus pesticides failed to show response.

Determination of OP insecticides using sodium hydroxide treated paper strip

The filter paper was initially spotted with 10 ppm standards of chlorpyrifos, dichlorvos, malathion, methyl parathion, monocrotophos, phosphamidon, profenofos, quinalphos and triazophos and then treated with sodium hydroxide solution. Among these pesticides only methyl parathion showed positive response by developing yellow colour. Methyl parathion was further spotted at

concentrations of 1, 5 and 10 ppm on the reagent treated filter paper and the yellow colour development was seen upto 1 ppm. The darkness of the coloured spot increased with increased levels of concentration. The colour development due to methyl parathion is depicted in plate 1.

Determination of OP insecticides using silver nitrate and sodium hydroxide treated paper strip

The silver nitrate treated filter paper was initially spotted with 10 ppm standards of chlorpyrifos, dichlorvos, malathion, methyl parathion, monocrotophos, phosphamidon, profenofos, quinalphos and triazophos. Then the pesticide spotted filter paper was treated with sodium hydroxide. The results shown that among different pesticides, phosphamidon and quinalphos showed positive response by developing colour.

Phosphamidon was further spotted on the silver nitrate treated filter paper at different concentrations and treated with sodium hydroxide revealed brown spots. Light brown colour spot was observed at the lowest concentration of 2 ppm and dark brown colour spot was observed in highest concentration of 10 ppm. The darkness of the spot was increased with increased concentration. The colour development due to phosphamidon is illustrated in plate 2.

Quinalphos was further spotted on the silver nitrate treated filter paper at different concentrations and treated with sodium hydroxide revealed white spots. Light colour spot was observed at the lowest concentration of 2 ppm and dark colour spot was observed in highest concentration of 10 ppm. The darkness of the spot was increased with increased concentration. The coloured spot of quinalphos is illustrated in plate 3.

Determination of OP insecticides using methyl red treated paper strip

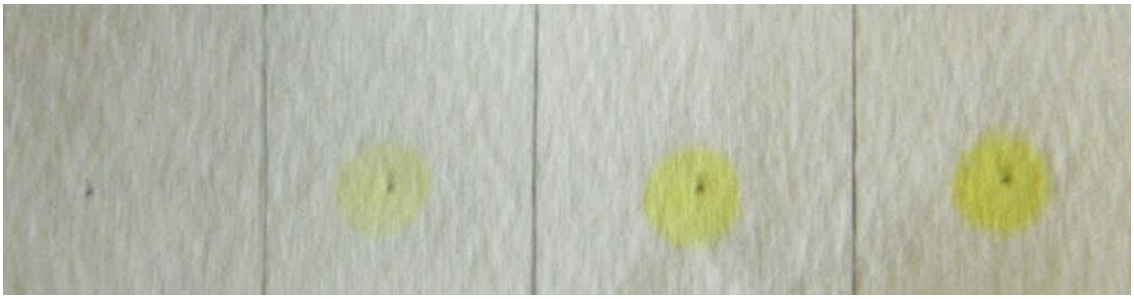
The methyl red treated filter paper wasspotted with 10 ppm standards of chlorpyrifos, dichlorvos, malathion, methyl parathion, monocrotophos, phosphamidon, profenofos, quinalphos and triazophos.

None of organophosphorus pesticides showed colour development.

Determination of OP insecticides using sodium carbonate and methanolic ferric chloride treated paper strip

The sodium carbonate and methanolic reagent treated filter paper was initially spotted with 10 ppm standards of chlorpyrifos, dichlorvos, malathion, methyl parathion, monocrotophos, phosphamidon, profenofos, quinalphos and triazophos. None of the pesticides showed response for colour development.

Plate.1 Response of methyl parathion in sodium hydroxide treated paper strip



Control	1 ppm	5 ppm	10 ppm

Plate.2 Response of phosphamidon in silver nitrate and sodium hydroxide treated paper strip

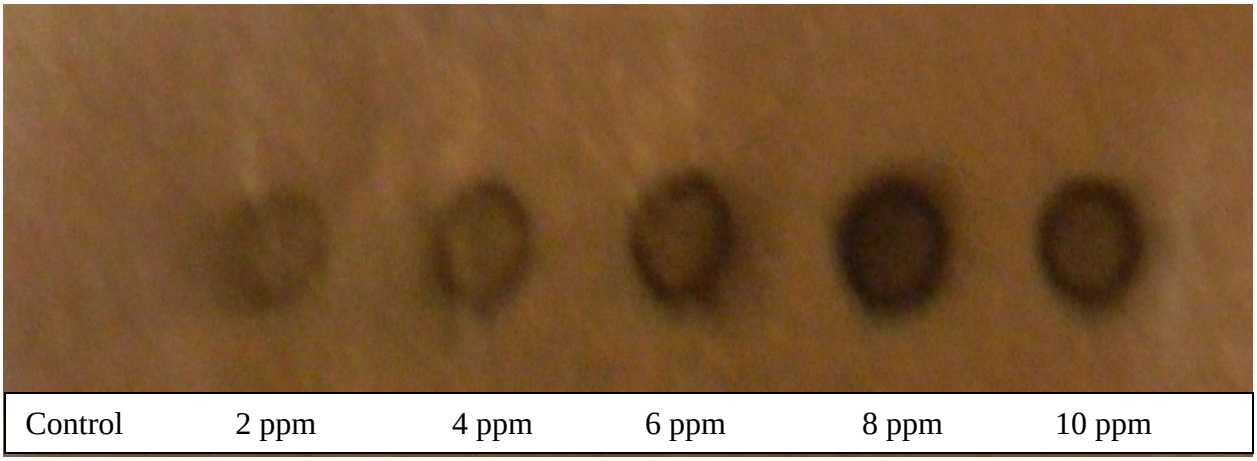


Plate.3 Response of quinalphos in silver nitrate and sodium hydroxide treated paper strip

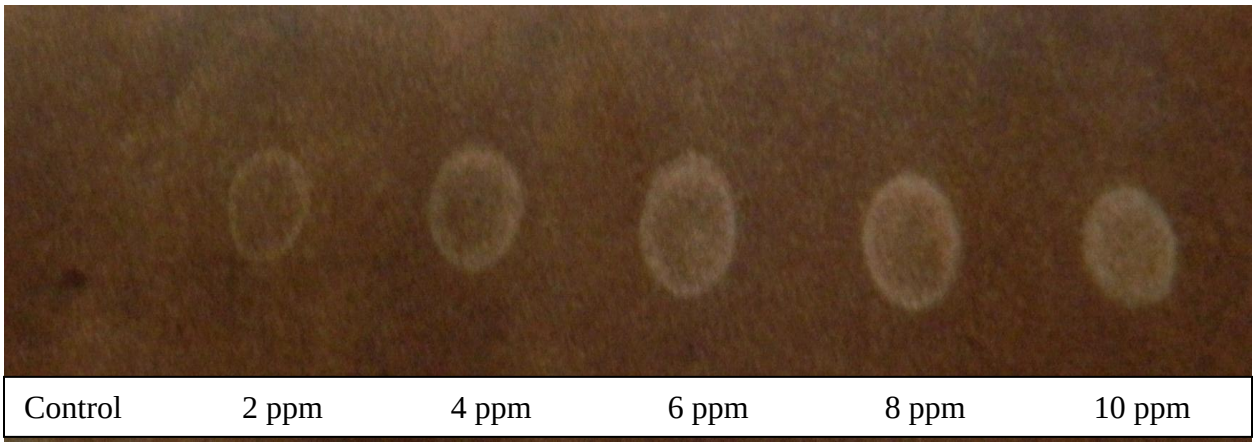
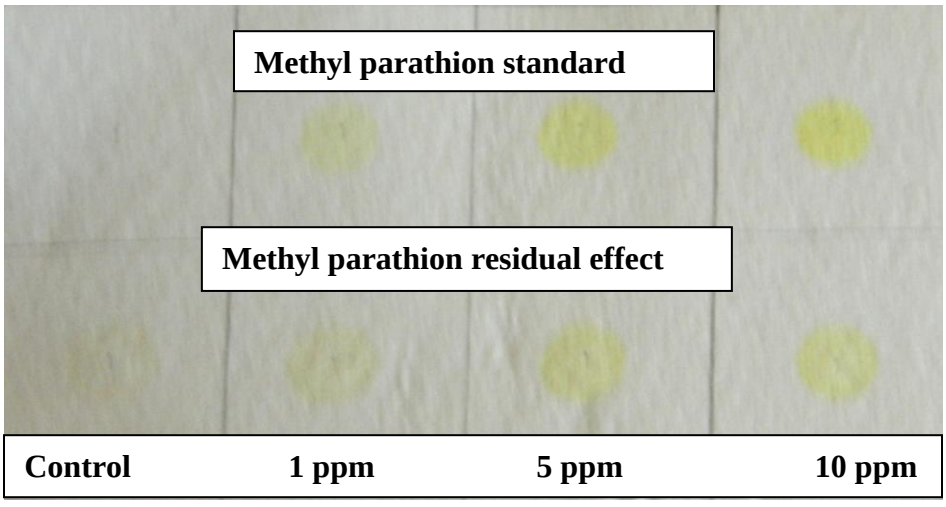


Plate.4 Response of methyl parathion standard and residual extract in paper strip treated with sodium hydroxide



Determination of pesticides in fortified vegetable

Determination of methyl parathion in fortified vegetable

Methyl parathion was fortified on to the vegetable sample at different concentrations (1, 5 and 10 ppm). The extract of methyl parathion were spotted on filter paper and treated with sodium hydroxide. The development of yellow colour on the spot was observed. The development of colour was not seen in control. The developed colour in the paper strip was comparable with standards.

Determination of phosphamidon and quinalphos in fortified vegetable

Phosphamidon was fortified in vegetable at different concentrations (2, 4, 6, 8 and 10 ppm). Phosphamidon residues extract were spotted on the silver nitrate solution treated filter paper. The interference of the vegetable matrices both control and the residual extract reacted with silver nitrate and produced black colour which is not comparable with brown colour of phosphamidon standards.

Quinalphos was fortified in vegetable at different concentrations (2, 4, 6, 8 and 10 ppm). Quinalphos residues extract were spotted on the silver nitrate solution treated filter paper. The interference of the vegetable matrices both control and the residual extract reacted with silver nitrate and produced black colour which is not comparable with white colour of quinalphos standards.

Validation of methyl parathion

Methyl parathion standards were fortified on to the vegetable at the concentration of 1, 5 and 10 ppm. Standard of methyl parathion and sample extract were spotted on the filter paper and treated with sodium hydroxide. The development of yellow colour was observed both in standards and samples at different concentration and is represented in plate 4.

Monitoring of methyl parathion residues in market samples using chromogenic reagent treated paper strip

Bhendi, brinjal, cabbage and cauliflower vegetable sample collected from four different markets at weekly intervals were analysed for methyl parathion residues present in the sample through the chromogenic reagent treated paper strip. The presence of methyl parathion residue was not observed in vegetable sample collected from different places and different dates.

The OP pesticides *viz.*, chlorpyrifos, dichlorvos, malathion, methyl parathion, monocrotophos, phosphamidon, profenofos, quinalphos and triazophos were spotted with iodine and silver nitrate treated filter paper for evaluation. Among the different pesticides tried methyl parathion showed positive response by developing yellow colour spots.

The darkness of the colour on the spot increased with increasing concentrations of the pesticides. Similar to the present findings Katrolia *et al.*, (1973) observed different coloured spots when sprayed with silver nitrate, alcoholic sodium hydroxide and palladium chloride for several organophosphorus insecticides in biological material. The development of yellow colour was observed on the spot of methyl parathion standard and sample indicating the suitability of chromogenic reagent for methyl parathion detection in vegetables.

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