

Determination of Acephate, Chlorpyrifos and Malathion Residues in Tomato

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ABSTRACT: Over the last years, the detection of pesticide residues in the official food safety surveillance programs of India has been increased, mainly in fresh vegetables like tomatoes. The organophosphorus insecticides are frequently applied in tomato cultivation in India. The study was initiated to determine the acephate, chlorpyrifos and malathion residues in tomatoes after harvest with UV-VIS spectrophotometer. The fruit samples were collected two hours after spraying (HAS), 1, 3, 5, 7 and 10 days after spraying (DAS). The results of the present study revealed that the tomato fruit samples which were collected at 2 HAS, 1 and 3 DAS (1.464 and 5.911 mg kg⁻¹) showed the acephate and malathion residues to be above maximum residue limits (MRL's are 1 and 3 mg kg⁻¹) and below MRL at 5 DAS (0.319 and 1.586 mg kg⁻¹). Similarly, the chlorpyrifos residues were detected above MRL (0.5 mg kg⁻¹) in the fruit samples collected up to 5 DAS (1.278 mg kg⁻¹) and the subsequent samples collected at 7 and 10 DAS showed the residues below detectable limits (BDL).

Keywords: UV-VIS Spectrophotometer, acephate, chlorpyrifos, malathion and maximum residue limit.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is considered one of the most important and remunerative vegetable crops cultivated throughout the world owing to its high nutritive values. It is a major contributor to the fruits and vegetable diet of humans throughout the world (Kapasiya *et al.*, 2015). In India, it is the third most important vegetable crop next to potato and onion in terms of production (Ahluwalia, 2019), which is 20.58 MMt which comes from 812 thousand hectares of land with productivity of 25.33 Mt ha⁻¹ during 2019-2020. The major tomato growing states in India are Andhra Pradesh, Madhya Pradesh, Odisha, Karnataka, Telangana etc. in terms of production and productivity, Andhra Pradesh state stood first with 2.75 MMt and 44.50 Mt ha⁻¹ respectively surpassing Madhya Pradesh (Ministry of Agriculture and Farmers Welfare, 2019). Tomatoes, wherever grown, are hosts for many kinds of

insects. They can cause unthrifty growth or death of the tomato plant and damage to fruit in the form of scarring, tissue destruction, and aberrations in shape or colour. To control these polyphagous insect pests, to prevent pre and post-harvest losses and to provide sufficient nutritive food for the burgeoning world population, the farmers were using a different group of insecticides *viz.*, organophosphates (OP), carbamates, organochlorines, synthetic pyrethroids and neonicotinoids for over four decades (Kramer *et al.*, 2012). During the applications of pesticides in farms, factors such as frequency application, equipment, mixer conditions and exposure determine the potential chemical risks to human health (Maclachlan *et al.*, 2010). Pesticides have consistently revealed their worth through increased agriculture productivity, reduced insect-borne, endemic diseases and protection as well as restoration of plantations, forests, harvested wood

products, homes and fibre (Ecobichon, 2000). Currently, pesticides are very valuable in developing nations, particularly those in tropical areas looking for entry into the global economy by providing off-season fresh vegetables and fruits to nations in more temperate weather. However, these goals cannot be achieved without the increased use of pesticides, mainly insecticides, herbicides and fungicides (Ecobichon, 2001). Food products contaminated with toxic pesticides are associated with severe effects on human health. More than 95% of sprayed insecticides and herbicides reach a destination other than their target species, including non-target species, air, water and soil (Cooper and Dobson, 2007). Pesticide contamination of both surface and ground waters can affect aquatic animals and plants, as well as human health when water is used for public consumption (Cerejeira *et al.*, 2003). The increased use of chemical pesticides has resulted in contamination of the environment and also caused many associated long-term effects on human health. Pesticides have been associated with a wide spectrum of human health hazards, ranging from short-term impacts such as headaches and nausea to chronic impacts like cancer, reproductive harm and endocrine disruption (Bankar *et al.*, 2012). The use of pesticides helped to increase crop yield and improve product quality, but due to their indiscriminate usage, the residue levels of the pesticides increased in the economic parts of the vegetables (Aktar *et al.*, 2009). Food Safety and Standards Authority of India reported that among the 23,660 samples analysed, pesticide residues were detected in 4,510 samples (19.10%), out of which the residues in 523 (2.2%) were found exceeding maximum residue limits (MRL's) (FSSAI, 2019). It was found that 61 per cent of the 712 vegetable samples analysed contained pesticide residues, 11 per cent of which exceeded the respective MRL values (Pavan, 2010). To alleviate the problems that are created by the increased and indiscriminate use of pesticides, any tool that is good enough which meet the environmental needs, provides a good harvest to the farmer and safety to the people. Judicious use of chemicals as a component of integrated pest management (IPM) is the safest means of pest control among the methods available now. In this context, a study was conducted to determine the acephate, chlorpyrifos and malathion residues in tomatoes which were the most frequently used insecticides by tomato farmers in Andhra Pradesh.

MATERIALS AND METHODS

Insecticide residues were estimated in the tomato fruit samples using UV- VIS spectrophotometer based on Beer-Lambert's law. The harvestable tomato fruit samples from the plots sprayed with acephate, chlorpyrifos and malathion were collected at two hours after spraying (HAS), 1, 3, 5, 7 and 10 days after

spraying (DAS) respectively. A total of 108 tomato fruit samples were collected (3 samples from each treatment for six times after each spray) from the respective treatments and only three samples were collected from the control treatment for method validation.

Preparation of Primary Stock Solution. Acephate 75 SP, chlorpyrifos 20 EC and malathion 50 EC insecticides were bought from the local market to prepare insecticide standards. Different colouring reagents *i.e.*, anthracene, p-aminoacetophenone and ammonium metavanadate (purchased from Quality Traders, Guntur) were added while preparing the standards.

Primary stock solutions of acephate and chlorpyrifos at the concentration of 1000 $\mu\text{g ml}^{-1}$ each were prepared by adding exactly 0.13 g of acephate and 0.5 g of chlorpyrifos insecticides into 100 ml of double-distilled water. Malathion stock at the concentration of 1000 $\mu\text{g ml}^{-1}$ was prepared by adding 0.2 g of malathion in 100 ml of acetone solution for better solubility. Micropipettes, volumetric flasks and graduated test tubes were used to transfer the aliquots and to make up the volume throughout the experiment to reduce the error.

Preparation of Working Standards for Acephate Residues Analysis. Based on Beer's-Lambert law, working standards of acephate at 0.1, 0.5, 1.0, 3.0 and 5.0 $\mu\text{g ml}^{-1}$ concentrations were prepared by serial dilution method using 1000 $\mu\text{g ml}^{-1}$ of acephate primary stock solution. Acephate residues were determined by following the method described by Elgailani and Alghamdi (2018). The absorption spectrum of products was obtained by the reaction of acephate with anthracene in nitric acid (HNO_3).

Preparation of Anthracene Reagent: About one gram of anthracene was dissolved in 100 ml of concentrated HNO_3 (minimum assay 68-70%). The solution was stirred until the anthracene was completely dissolved to give reddish-orange colour and filtered to remove any unwanted particles. Care should be taken while handling concentrated HNO_3 .

Preparation of Working Solutions of Acephate for Analysis with UV-VIS Spectrophotometer: About 2 ml each of the prepared working standards (0.1, 0.5, 1.0, 3.0 and 5.0 $\mu\text{g ml}^{-1}$) of acephate was transferred into a test tube and 1 ml of anthracene reagent (extra pure 99%) followed by 2 ml of NaOH (Purity 97%) solution (1.0 M) were added. The solutions were kept static for 5 min until the coloured complex (red or orange) was developed. The absorbance of the solutions was measured at 380 nm (λ_{max}) versus blank.

Preparation of Working Standards for Chlorpyrifos Residues Analysis. Working standards of chlorpyrifos in the range 0.07 to 5.00 $\mu\text{g ml}^{-1}$ that obeyed Beer's-Lambert law were prepared by serial dilution method using a primary stock solution of 1000

$\mu\text{g ml}^{-1}$. The residues were determined by following the method described by Thakur *et al.* (2017). This method was based on alkaline hydrolysis of chlorpyrifos to 1, 2, 4-trichloropyridine, followed by coupling with diazotized p-aminoacetophenone in an alkaline medium.

Preparation of diazotized p-aminoacetophenone:

About 3.18 g of p-aminoacetophenone (99% extra pure) was dissolved in 45 ml of concentrated HCl (Hydrochloric acid, 35-38% minimum assay), and then diluted to 500 ml with distilled water (solution I). This solution was kept in a glass stoppered volumetric flask and was stored in an icebox when not in use for its stability for 2 months. Then, about 4.5 g of NaNO_3 (Sodium nitrate, extra pure 99%) was dissolved in 100 ml with distilled water (solution II). Thereafter, 2 ml of solution I was measured in a 25 ml brown glass stoppered graduate tube, placed in an ice bath and then 2 ml of solution II was added. The graduate tube was allowed to stand for 20 min with the occasional whirling of the tube. With the completion of this reaction, the diazo reagent was ready to use and was used within an hour after preparation.

Preparation of Final Working Solutions of Chlorpyrifos for Analysis with UV-VIS Spectrophotometer:

About 5 ml of the chlorpyrifos standard was taken in a 25 ml graduated tube and to this, 1 ml of 1 M sodium hydroxide (NaOH) (97% purity) was added. The solution was kept for 30 min at room temperature for complete hydrolysis. Then 1 ml of diazotized p-aminoacetophenone was added. Later, the solutions were kept for 45 min for yellowish-orange colour development. The solution was then diluted to the mark with distilled water in 25 ml graduated tube and absorbance was measured at 290 nm (λ_{max}) against a reagent blank.

Preparation of Working Standards for Malathion Residues Analysis.

Working standards of malathion ranged from 0.01 to 5.0 $\mu\text{g ml}^{-1}$ (Which obeyed Beer's-Lambert law) concentrations were prepared by serial dilution method using 1000 $\mu\text{g ml}^{-1}$ primary stock solution. Malathion residues were determined by following the method described by Norris *et al.* (1954) and Venugopal *et al.* (2012). Decomposition of malathion was done with the addition of alcoholic KOH (Potassium Hydroxide). Dimethyldithiophosphate produced was made to react with ammonium metavanadate in nitric acid that led to the formation of blue colour.

Preparation of Ammonium Metavanadate (NH_4VO_3) Reagent:

About 1.25 g of ammonium metavanadate was dissolved in hot water. The solution was cooled and 10 ml of concentrated HNO_3 (Nitric acid, minimum assay 68-70%) was added. The solution was allowed to stand overnight and filtered if necessary. Later, the solution was diluted to 500 ml with distilled water and was stored in a glass container.

Preparation of Working Solutions of Malathion for Analysis with UV-VIS Spectrophotometer:

An aliquot of 2 ml malathion working standard, 2 ml of ammonium metavanadate (98% extra pure), 1ml of potassium hydroxide (99.99% pure) and 2 ml of ethyl alcohol (99.50% pure) were taken into a 25 ml standard flask and volume was made up to 25 ml with concentrated HNO_3 and with water. The resultant mixture was heated at 50-70°C for one hour until the solution turned blue. The resulting absorbance of the blue colour was measured at 760 nm (λ_{max}).

Extraction and Clean-up of Samples.

A representative tomato fruit sample of 50 g was collected from the pooled replications of respective treatments of acephate, chlorpyrifos and malathion in the field. About 5-10 g of anhydrous sodium sulphate (Na_2SO_4) (99.3-99.7% pure) was added to the fruit sample and then it was made into a fine paste using a Sumeet Mixer grinder (blending machine). Then, the blended sample was filtered using the Buchner funnel under suction using Sisco[®] vacuum pump. The filtrate was later transferred to one litre separatory funnel and extracted with 100 ml each of ethyl acetate (99% purity) and dichloromethane (99.8% purity) (exchange solvent) by vigorous shaking. The separatory funnel was kept standstill for the formation of organic and aqueous layers depending on a density gradient. The organic layer of each of the samples was collected in a conical flask and the upper aqueous layer was discarded. Again, ethyl acetate and dichloromethane solvents were added to the aqueous layer in the separatory funnel and shaken well. Further, the lower organic layer was collected. The organic layers thus collected were filtered with Whatman no.1 filter paper after the addition of 15 g of Na_2SO_4 . The elute was later concentrated up to 5-10 ml using a rotary vacuum evaporator at a temperature of 50-60°C and 240-250 rpm.

Preparation of Tomato Fruit Samples for Analysis with UV-VIS Spectrophotometer.

About 5 ml of the concentrated tomato fruit sample elute was taken in a test tube, and 2 ml each of the respective colouring reagents of insecticides were added. The absorbance values and wavelength scans were taken using a UV-VIS spectrophotometer after the colour development in the tomato fruit sample at wavelengths 380, 260 and 760 nm for acephate, chlorpyrifos and malathion, respectively.

Estimation of Insecticide Residues in UV-VIS Spectrophotometer.

A wavelength scan of the working solutions was done to ensure the elution of the peak at the specific wavelength. About 5 ml each of acephate (red or orange coloured), chlorpyrifos (yellowish-orange coloured) and malathion (blue coloured) working solutions was taken into each quartz cuvettes and scanned to determine the absorption maxima (λ_{max}). Further, the absorbance values of working solutions were taken and a calibration graph was plotted between

absorbance and concentration to check the linearity of the standards.

The tomato fruit samples that were prepared as mentioned above were scanned to confirm the presence of residue at that particular absorption maximum (λ_{\max}) as obtained for the insecticide working solutions. Further, the absorbance values were taken at the absorption maxima (λ_{\max}) for the fruit samples from the respective acephate, chlorpyrifos and malathion treatments.

A regression equation “ $y = bx + C$ ” was developed from the linearity graph of standards, in which the absorbance values of the tomato fruit samples (3 replications in each treatment) elute was substituted to determine the respective insecticide residue in the field-collected tomato fruit samples. ‘y’ is the absorbance of the tomato fruit sample, ‘b’ is the slope of the linearity graph of the insecticide standards, ‘x’ is the insecticide residue value and c is the intercept.

Method Validation. The method was validated in terms of linearity, accuracy, precision (intraday and inter-day precision), sensitivity and repeatability.

Linearity: The aliquots of concentrations ranging from 0.1 to 5 $\mu\text{g ml}^{-1}$ for acephate, 0.07 to 5 $\mu\text{g ml}^{-1}$ for chlorpyrifos and 0.01 to 5 $\mu\text{g ml}^{-1}$ for malathion were analysed in triplicate. The results were used to calculate the equation of the line by using linear regression by the least-squares regression method.

Accuracy: To the pre analysed tomato fruit sample (control treatment) solutions, a known amount of standard insecticide stock solution was added at different levels *viz.*, 80, 100 and 120 per cent *i.e.*, 0.8, 1.0 and 1.2 $\mu\text{g ml}^{-1}$. These solutions were reanalysed by the proposed method and the recovery (%) was calculated by using the following formula.

$$\text{Recovery (\%)} = \frac{\text{Amount found}}{\text{Amount added}} \times 100$$

Precision: The precision of the method was studied as intraday and inter-day variations. Intraday precision was determined by analysing the particular concentrations *vs* absorbances of insecticide solutions in the spectrophotometer at three hours interval for three times on the same day. Inter day precision was determined by analysing the particular concentrations of insecticide solutions for three days over a week.

Repeatability: Repeatability expressed as relative standard deviation (RSD) was calculated by analysing the particular concentration of insecticide solution in terms of absorbances six times with no time-lapse. RSD was calculated by using the following formula.

$$\text{RSD} = \frac{\text{Standard deviation}}{\text{Mean}} \times 100$$

Sensitivity: The sensitivity measurements of insecticide residues were estimated in terms of the limit of quantification (LOQ) and limit of detection (LOD).

The LOQ and LOD were calculated by using the following equations.

$$\text{LOD} = 3.3 \times N/B$$

$$\text{LOQ} = 10 \times N/B$$

Where, ‘N’ is the standard deviation of blank absorbance values of the respective insecticide (n = 3), taken as a measure of noise, and ‘B’ is the slope of the corresponding calibration curve.

RESULTS AND DISCUSSION

Method validation. Linearity: Linearity graphs of acephate, chlorpyrifos and malathion insecticide standards were shown in Fig. 1, 2, 3. The concentrations and absorbance values of the respective insecticides were given in Tables 1, 2 and 3. It was seen that the concentration and absorbance were directly proportional to each other. Hence, Beers law was obeyed in the range 0.1 to 5 $\mu\text{g ml}^{-1}$ for acephate, 0.07 to 5 $\mu\text{g ml}^{-1}$ for chlorpyrifos and 0.01 to 5 $\mu\text{g ml}^{-1}$ for malathion.

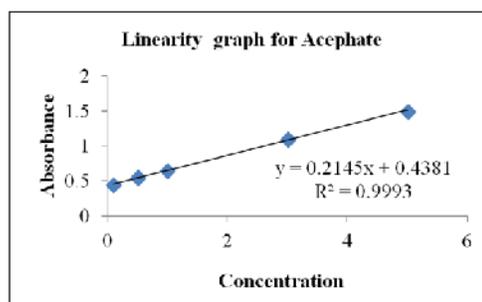


Fig. 1. Linearity graph for acephate standards.

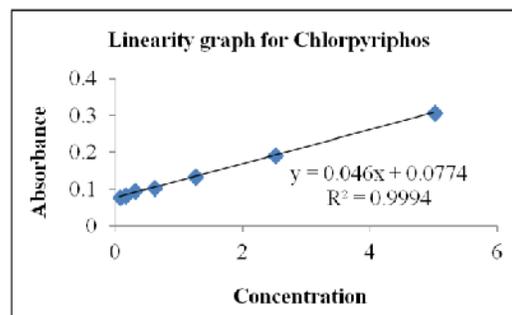


Fig. 2. Linearity graph for chlorpyrifos standards.

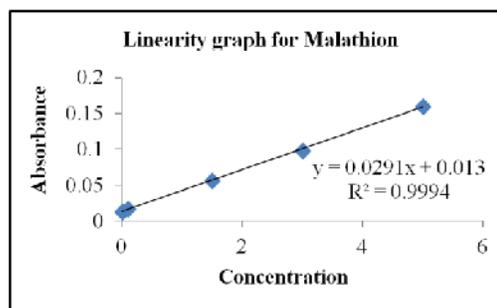


Fig. 3. Linearity graph for malathion standards.

Table 1: Concentration and absorbance values of acephate standards.

Concentration	Absorbance
0.1 $\mu\text{g ml}^{-1}$	0.45
0.5 $\mu\text{g ml}^{-1}$	0.55
1 $\mu\text{g ml}^{-1}$	0.65
3 $\mu\text{g ml}^{-1}$	1.1
5 $\mu\text{g ml}^{-1}$	1.5

Table 2: Concentration and absorbance values of chlorpyrifos standards.

Concentration	Absorbance
0.07 $\mu\text{g ml}^{-1}$	0.078
0.15 $\mu\text{g ml}^{-1}$	0.083
0.3 $\mu\text{g ml}^{-1}$	0.095
0.6 $\mu\text{g ml}^{-1}$	0.104
1.25 $\mu\text{g ml}^{-1}$	0.135
2.5 $\mu\text{g ml}^{-1}$	0.192
5 $\mu\text{g ml}^{-1}$	0.307

Table 3: Concentration and absorbance values of malathion standards.

Concentration	Absorbance
0.01 $\mu\text{g ml}^{-1}$	0.013
0.1 $\mu\text{g ml}^{-1}$	0.017
1.5 $\mu\text{g ml}^{-1}$	0.057
3 $\mu\text{g ml}^{-1}$	0.098
5 $\mu\text{g ml}^{-1}$	0.160

Table 4: Recovery of acephate from the tomato control samples.

Fortification level ($\mu\text{g ml}^{-1}$)	Amount recovered ($\mu\text{g ml}^{-1}$)	Recovery (%)	SD	% RSD
0.8	0.793	99.09	0.001	0.19
1	0.984	98.35	0.017	1.75
1.2	1.190	98.89	0.014	1.14

Table 5: Recovery of chlorpyrifos from the tomato control samples

Fortification level ($\mu\text{g ml}^{-1}$)	Amount recovered ($\mu\text{g ml}^{-1}$)	Recovery (%)	SD	% RSD
0.8	0.781	97.64	0.012	1.61
1.0	0.977	97.68	0.013	1.28
1.2	1.179	98.25	0.012	1.02

SD = Standard deviation; % RSD = % Relative Standard Deviation

Table 6: Recovery of malathion from the tomato control samples.

Fortification level ($\mu\text{g ml}^{-1}$)	Amount recovered ($\mu\text{g ml}^{-1}$)	Recovery (%)	SD	% RSD
0.8	0.782	97.723	0.015	1.90
1.0	0.989	98.855	0.009	0.87
1.2	1.174	97.843	0.022	1.86

SD = Standard deviation; % RSD = % Relative Standard Deviation

Table 7: Intraday precision results of acephate.

Concentration	Abs @ 10 AM	Abs @ 1 PM	Abs @ 4 PM	Mean Abs	SD	% RSD
0.1 $\mu\text{g ml}^{-1}$	0.450	0.460	0.448	0.453	0.006	1.419
0.5 $\mu\text{g ml}^{-1}$	0.550	0.560	0.548	0.553	0.006	1.163
1 $\mu\text{g ml}^{-1}$	0.650	0.640	0.655	0.648	0.008	1.179
3 $\mu\text{g ml}^{-1}$	1.100	1.080	1.120	1.100	0.020	1.818
5 $\mu\text{g ml}^{-1}$	1.500	1.470	1.450	1.473	0.025	1.708

Abs = Absorbance; SD = Standard deviation; % RSD = % Relative Standard Deviation

Accuracy: Recovery (%) values of acephate, chlorpyrifos and malathion in fortified samples were presented in Tables 4-6. Recovery per cent values for acephate (98.35 to 99.09 %), chlorpyrifos (97.64 to 98.25 %) and malathion (97.72 to 98.85 %) at different fortification levels were in the acceptable range. The relative standard deviation (% RSD) values for all the three-insecticide fortified tomato fruit samples were less than two per cent. Therefore, the method followed was simple and accurate.

Precision: Intraday and inter-day absorbance values of acephate, chlorpyrifos and malathion insecticide standards with % RSD less than two per cent indicated the method followed was very precise (Table 7-12).

Sensitivity: The sensitivity measurements of insecticide residues were estimated in terms of LOD and LOQ which were presented for acephate, chlorpyrifos and malathion insecticides in Table 13, where it can be clearly understood that even lower levels of insecticide residues can be estimated with help of UV- VIS spectrophotometer. These results were in line with the findings of Reddy *et al.* (2005); Venugopal *et al.* (2012) where the LOD values of malathion were found to be 0.052 and 0.13, respectively.

Table 8: Inter-day precision results of acephate.

Concentration	Abs @ Day 1	Abs @ Day 2	Abs @ Day 3	Mean Abs	SD	% RSD
0.1 $\mu\text{g ml}^{-1}$	0.450	0.458	0.448	0.452	0.005	1.171
0.5 $\mu\text{g ml}^{-1}$	0.550	0.565	0.548	0.554	0.009	1.676
1 $\mu\text{g ml}^{-1}$	0.650	0.665	0.655	0.657	0.008	1.163
3 $\mu\text{g ml}^{-1}$	1.100	1.130	1.120	1.117	0.015	1.368
5 $\mu\text{g ml}^{-1}$	1.500	1.501	1.450	1.484	0.029	1.965

Abs = Absorbance; SD = Standard deviation; % RSD = % Relative Standard Deviation

Table 9: Intraday precision results of chlorpyrifos.

Concentration	Abs @ 10 AM	Abs @ 1 PM	Abs @ 4 PM	Mean Abs	SD	% RSD
0.07 $\mu\text{g ml}^{-1}$	0.078	0.080	0.078	0.079	0.001	1.187
0.15 $\mu\text{g ml}^{-1}$	0.083	0.085	0.083	0.084	0.001	1.122
0.3 $\mu\text{g ml}^{-1}$	0.095	0.097	0.095	0.096	0.001	0.981
0.6 $\mu\text{g ml}^{-1}$	0.104	0.107	0.105	0.105	0.001	1.355
1.25 $\mu\text{g ml}^{-1}$	0.135	0.139	0.135	0.136	0.002	1.615
2.5 $\mu\text{g ml}^{-1}$	0.192	0.198	0.198	0.196	0.003	1.678
5 $\mu\text{g ml}^{-1}$	0.307	0.314	0.310	0.310	0.003	1.122

Abs = Absorbance; SD = Standard deviation; % RSD = % Relative Standard Deviation

Table 10: Inter-day precision results of chlorpyrifos.

Concentration	Abs @ Day 1	Abs @ Day 2	Abs @ Day 3	Mean Abs	SD	% RSD
0.15 $\mu\text{g ml}^{-1}$	0.083	0.086	0.083	0.084	0.001	1.478
0.3 $\mu\text{g ml}^{-1}$	0.095	0.099	0.095	0.096	0.002	1.917
0.6 $\mu\text{g ml}^{-1}$	0.104	0.107	0.104	0.105	0.002	1.537
1.25 $\mu\text{g ml}^{-1}$	0.135	0.138	0.134	0.136	0.002	1.592
2.5 $\mu\text{g ml}^{-1}$	0.192	0.199	0.197	0.196	0.003	1.754
5 $\mu\text{g ml}^{-1}$	0.307	0.317	0.309	0.311	0.005	1.580

Abs = Absorbance; SD = Standard deviation; % RSD = % Relative Standard Deviation

Table 11: Intraday precision results of malathion.

Concentration	Abs @ 10 AM	Abs @ 1 PM	Abs @ 4 PM	Mean Abs	SD	% RSD
0.01 $\mu\text{g ml}^{-1}$	0.013	0.013	0.013	0.013	0.0002	1.671
0.10 $\mu\text{g ml}^{-1}$	0.017	0.017	0.017	0.017	0.0002	1.455
1.50 $\mu\text{g ml}^{-1}$	0.057	0.058	0.056	0.057	0.0008	1.433
3.00 $\mu\text{g ml}^{-1}$	0.098	0.098	0.100	0.098	0.0011	1.133
5.00 $\mu\text{g ml}^{-1}$	0.160	0.166	0.165	0.163	0.0031	1.887

Abs = Absorbance; SD = Standard deviation; % RSD = % Relative Standard Deviation

Table 12: Inter-day precision results of malathion.

Concentration	Ab @ Day 1	Abs @ Day 2	Abs @ Day 3	Mean Abs	SD	% RSD
0.01 $\mu\text{g ml}^{-1}$	0.013	0.013	0.013	0.013	0.0003	1.989
0.10 $\mu\text{g ml}^{-1}$	0.017	0.017	0.017	0.017	0.0003	1.499
1.50 $\mu\text{g ml}^{-1}$	0.057	0.058	0.057	0.057	0.0005	0.859
3.00 $\mu\text{g ml}^{-1}$	0.098	0.098	0.100	0.099	0.0011	1.126
5.00 $\mu\text{g ml}^{-1}$	0.160	0.160	0.165	0.162	0.0032	1.968

Abs = Absorbance; SD = Standard deviation; % RSD = % Relative Standard Deviation

Table 13: Sensitivity measurements for acephate, chlorpyriphos and malathion insecticide residues.

Sr. No.	Insecticide	Blank Absorbance			Mean	SD	LOD	LOQ
		I	II	III				
1.	Acephate	0.4265	0.4260	0.4268	0.4264	0.0004	0.0062	0.0188
2.	Chlorpyriphos	0.0001	0.0012	0.00105	0.0008	0.0006	0.0428	0.1296
3.	Malathion	0.0054	0.00565	0.00552	0.0055	0.0001	0.0143	0.0432

SD = Standard deviation; LOD = Limit of detection; LOQ = Limit of quantification

Repeatability: Absorbance values of the respective insecticide standards (Tables 14, 15) were taken six times repeatedly with % RSD less than two per cent confirmed the method to be accurate and precise.

Table 14: Repeatability results of acephate.

Concentration	Abs I	Abs II	Abs III	Abs IV	Abs V	Abs VI	Mean Abs	SD	% RSD
0.5 $\mu\text{g ml}^{-1}$	0.550	0.560	0.548	0.550	0.565	0.548	0.554	0.007	1.302
1 $\mu\text{g ml}^{-1}$	0.650	0.640	0.655	0.650	0.665	0.655	0.653	0.008	1.259
3 $\mu\text{g ml}^{-1}$	1.100	1.080	1.120	1.100	1.130	1.120	1.108	0.018	1.656
5 $\mu\text{g ml}^{-1}$	1.500	1.470	1.450	1.500	1.501	1.450	1.479	0.025	1.692

Abs = Absorbance; SD = Standard deviation; % RSD = % Relative Standard Deviation

Table 15: Repeatability results of chlorpyriphos.

Concentration	Abs I	Abs II	Abs III	Abs IV	Abs V	Abs VI	Mean Abs	SD	% RSD
0.15 $\mu\text{g ml}^{-1}$	0.083	0.085	0.083	0.083	0.085	0.083	0.084	0.001	1.003
0.3 $\mu\text{g ml}^{-1}$	0.095	0.097	0.095	0.095	0.097	0.095	0.096	0.001	0.878
0.6 $\mu\text{g ml}^{-1}$	0.104	0.107	0.105	0.104	0.107	0.105	0.105	0.001	1.212
1.25 $\mu\text{g ml}^{-1}$	0.135	0.139	0.135	0.135	0.139	0.135	0.136	0.002	1.445
2.5 $\mu\text{g ml}^{-1}$	0.192	0.198	0.198	0.192	0.198	0.198	0.196	0.003	1.501
5 $\mu\text{g ml}^{-1}$	0.307	0.314	0.310	0.307	0.314	0.310	0.310	0.003	1.003

Abs = Absorbance; SD = Standard deviation; % RSD = % Relative Standard Deviation

Table 16: Repeatability results of malathion.

Concentration	Abs I	Abs II	Abs III	Abs IV	Abs V	Abs VI	Mean Abs	SD	% RSD
0.01 $\mu\text{g ml}^{-1}$	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.0002	1.644
0.1 $\mu\text{g ml}^{-1}$	0.017	0.017	0.017	0.017	0.017	0.017	0.017	0.0002	1.345
1.5 $\mu\text{g ml}^{-1}$	0.057	0.058	0.056	0.057	0.058	0.057	0.057	0.0006	1.064
3 $\mu\text{g ml}^{-1}$	0.098	0.098	0.100	0.098	0.098	0.100	0.098	0.0010	1.014
5 $\mu\text{g ml}^{-1}$	0.160	0.166	0.165	0.160	0.160	0.165	0.163	0.0030	1.817

Abs = Absorbance; SD = Standard deviation; % RSD = % Relative Standard Deviation

Determination of Acephate Residues with Spectrophotometer. The wavelength scans of acephate working standards and the tomato fruit samples collected at the 2 HAS, 1, 3 and 5 DAS were

depicted in Fig. 4-8. Wavelength scan graphs of tomato fruit samples showed peaks near 380 nm that coincided with that of the standards reference graph of the acephate.

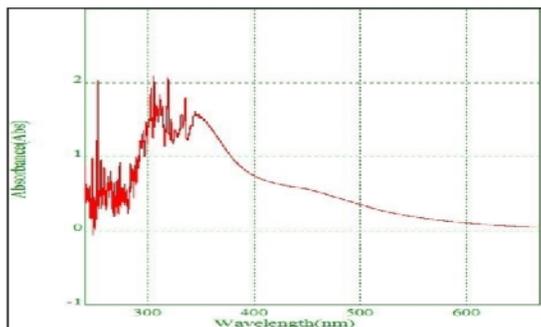


Fig. 4. Wavelength scan of acephate standards.

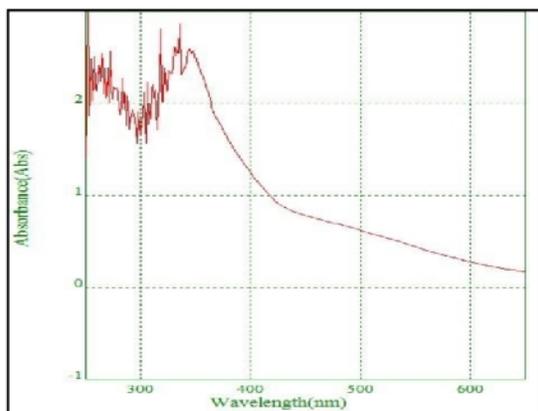


Fig. 5. Wavelength scan of tomato fruit sample from acephate treatment at 2 HAS.

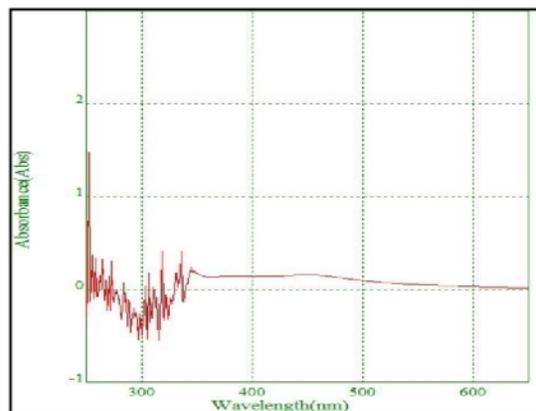


Fig. 7. Wavelength scan of tomato fruit sample from acephate treatment at 3 DAS.

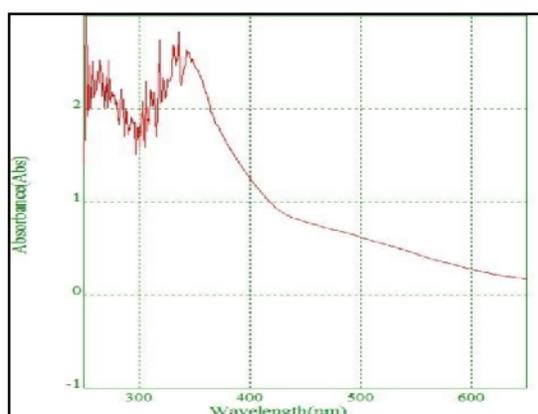


Fig. 6. Wavelength scan of tomato fruit sample from acephate treatment at 1 DAS.

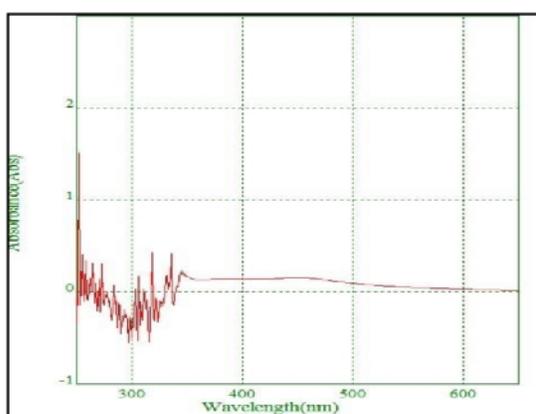


Fig. 8. Wavelength scan of tomato fruit sample from acephate treatment at 5 DAS.

The residue values obtained from the regression equation of acephate were presented in Table 17. The residues at two HAS, 1, 3, and 5 DAS were found to be 5.426, 4.376, 1.464, 0.319 mg kg⁻¹ respectively. The residues in the samples collected at the 7 and 10 DAS were found to be at BDL, where the limit of detection was 0.0062 (Table 13).

The MRL for acephate in tomato as per Codex Alimentarius Commission (CAC, 2009) was 1 mg kg⁻¹ whereas no MRL was given for acephate in tomato by

the Food Safety Standards Authority of India (FSSAI, 2019). As the tomato samples which were collected at 5 DAS have residues below MRL, a minimum of five days safe waiting period may be required for the acephate residues to decline below MRL. These results were in line with the findings of Singh *et al.* (2019) who stated that acephate residues on tomato and cucumber were reduced to half in less than two days with a safe waiting period of nine days.

Table 17: Residues of acephate in the tomato samples.

Interval (Days)	Residues (mg kg ⁻¹)				MRL (1 mg kg ⁻¹)
	R ₁	R ₂	R ₃	Mean ± SD	
0	5.430	5.429	5.420	5.426 ± 0.006	Above MRL
1	4.380	4.377	4.370	4.376 ± 0.005	Above MRL
3	1.469	1.465	1.458	1.464 ± 0.006	Above MRL
5	0.323	0.315	0.318	0.319 ± 0.004	Below MRL
7	BDL	BDL	BDL		
10	BDL	BDL	BDL		

MRL = Maximum Residue Limit as per Codex Alimentarius Commission (CAC); BDL = Below Detectable Limits

Determination of Chlorpyrifos Residues with Spectrophotometer. In Fig. 9-14, wavelength scan graphs of chlorpyrifos working standards and tomato fruit samples collected at the 2 HAS, 1, 3, 5 and 7 DAS were indicated. The peak absorbance was noticed near 290 nm that corresponded with that of the standards reference graph of the chlorpyrifos.

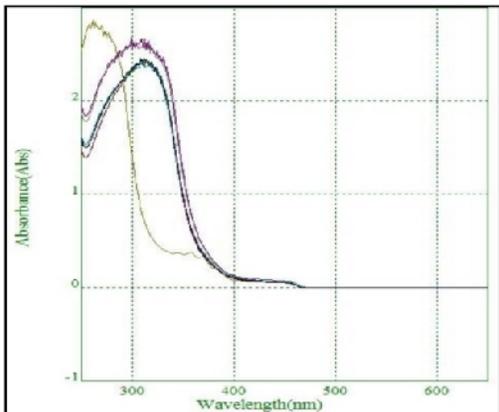


Fig. 9. Wavelength scan of chlorpyrifos standards.

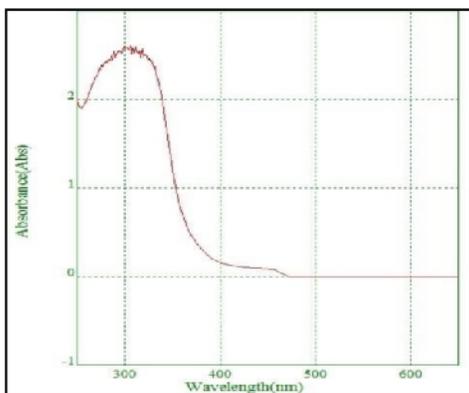


Fig. 10. Wavelength scan of tomato fruit sample from chlorpyrifos treatment at 2 HAS.

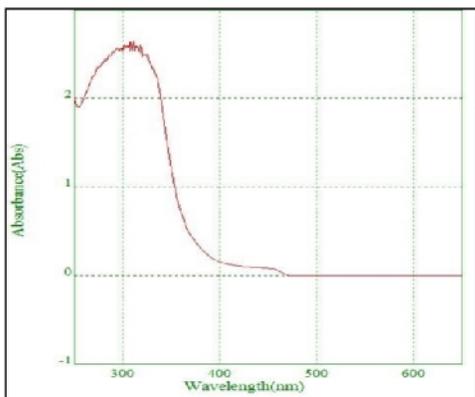


Fig. 11. Wavelength scan of tomato fruit sample from chlorpyrifos treatment at 1 DAS.

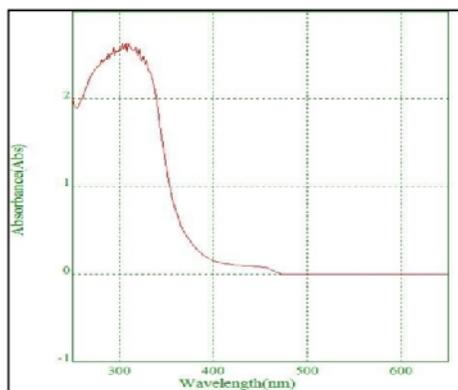


Fig. 12. Wavelength scan of tomato fruit sample from chlorpyrifos treatment at 3 DAS.

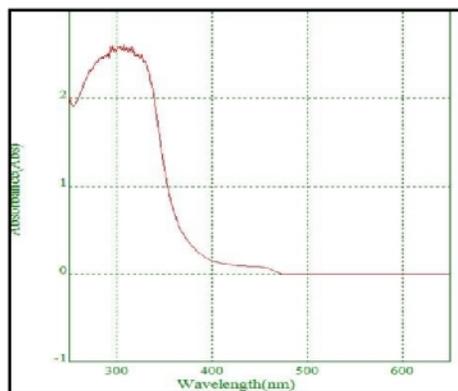


Fig. 13. Wavelength scan of tomato fruit sample from chlorpyrifos treatment at 5 DAS.

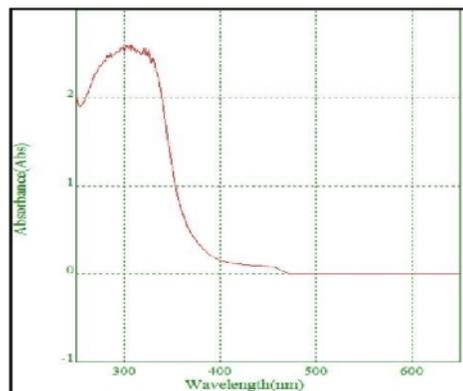


Fig. 14. Wavelength scan of tomato fruit sample from chlorpyrifos treatment at 7 DAS.

The chlorpyrifos residues values obtained from the regression equation at 2 HAS, 1, 3, 5, and 7 DAS were found to be 6.846, 5.390, 4.469, 1.279 and 0.286 mg kg⁻¹, respectively (Table 17). The residues in the samples collected at 10 DAS had negligible quantities *i.e.*, below detectable limits (BDL) where the LOD for chlorpyrifos was 0.043 (Table 13).

The maximum residue limit for chlorpyrifos as per the Food Safety Standards Authority of India (FSSAI, 2019) is 0.5 mg kg⁻¹. The samples which were collected at 7 DAS showed residues below MRL and those collected at 10 DAS showed the residues below detectable limits. Therefore, a safe waiting period of minimum seven days may be required for chlorpyrifos to decline below MRL in tomatoes. These results were

in line with the findings of Singh *et al.*, (2019) where the chlorpyrifos residues on tomato and cucumber were reduced to half in less than two days with a safe waiting period 10 days. Spectrophotometric analysis done by Thakur *et al.* (2017); Joshi *et al.* (2017) in cauliflower and tomato samples showed chlorpyrifos residues in the range of 2.93 to 6.83 mg kg⁻¹ and these results were in line with the above findings.

Table 18: Residues of chlorpyrifos in the tomato samples.

Interval (Days)	Residues (mg kg ⁻¹)				MRL (0.5 mg kg ⁻¹)
	R ₁	R ₂	R ₃	Mean ± SD	
0	6.850	6.848	6.839	6.846 ± 0.005	Above MRL
1	5.395	5.383	5.394	5.390 ± 0.006	Above MRL
3	4.470	4.470	4.467	4.469 ± 0.0013	Above MRL
5	1.280	1.278	1.278	1.279 ± 0.0010	Above MRL
7	0.287	0.287	0.286	0.286 ± 0.0005	Below MRL
10	BDL	BDL	BDL		

MRL = Maximum Residue Limit as per Food Safety Standards Authority of India (FSSAI); BDL = Below Detectable Limits

Determination of Malathion Residues with Spectrophotometer. The wavelength scans of the malathion working standards and fruit samples collected at the 2 HAS, 1, 3 and 5 DAS were depicted in Fig. 15-19. The wavelength scan graphs of tomato fruit samples showed peak absorbance near 760 nm and were matched with that of the standards reference graph (Fig. 15) of the malathion.

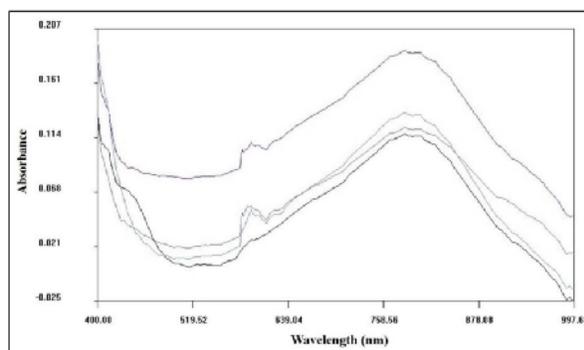


Fig. 15. Wavelength scan of malathion standards.

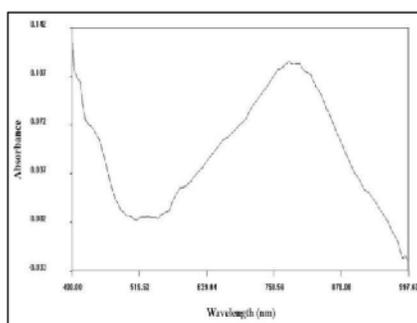


Fig. 16. Wavelength scan of tomato fruit sample from malathion treatment at 2 HAS.

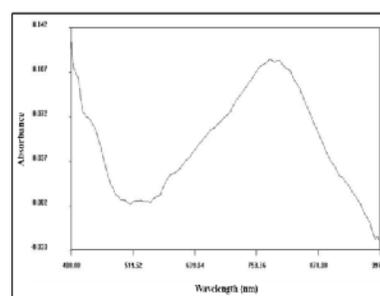


Fig. 17. Wavelength scan of tomato fruit sample from malathion treatment at 1 DAS.

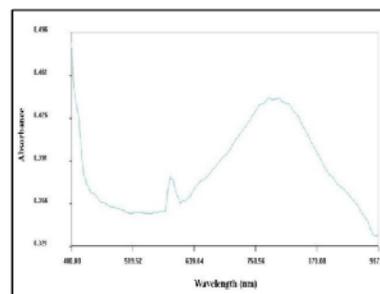


Fig. 18. Wavelength scan of tomato fruit sample from malathion treatment at 3 DAS.

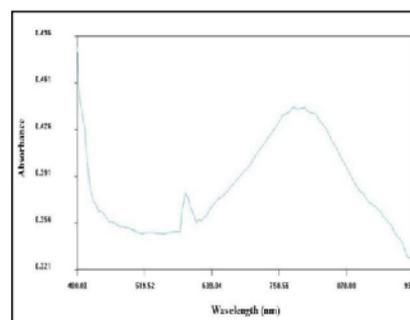


Fig. 19. Wavelength scan of tomato fruit sample from malathion treatment at 5 DAS.

The MRL for malathion in tomatoes as per the Food Safety Standards Authority of India (FSSAI, 2019) is 3 mg kg⁻¹. The samples which were collected at 2 HAS, 1, 3 and 5 DAS (Table 18) showed residues of 8.465, 7.112, 5.911 and 1.586 mg kg⁻¹ respectively. Similarly, the samples collected at 7 and 10 DAS showed the malathion levels below detectable limits where the LOD for malathion was 0.014 (Table 13). Therefore, a safe waiting period of five days may be required for malathion to decline below MRL in tomatoes. These

results were in line with the findings of Paneru *et al.* (2012) where the malathion residues on tomato and cauliflower were reduced to half within three days provided with a safe waiting period of five days. Analysis of malathion in cauliflower, cabbage and spinach samples were done by Mathew *et al.* (2007), Gouda *et al.* (2010); Tiwari and Asthana (2012); Venugopal *et al.*, (2012); Pandey *et al.* (2014) detected residues in the range of 1.256 to 11 mg kg⁻¹.

Table 18: Residues of chlorpyrifos in the tomato samples.

Interval (Days)	Residues (mg kg ⁻¹)				MRL (0.5 mg kg ⁻¹)
	R ₁	R ₂	R ₃	Mean ± SD	
0	6.850	6.848	6.839	6.846 ± 0.005	Above MRL
1	5.395	5.383	5.394	5.390 ± 0.006	Above MRL
3	4.470	4.470	4.467	4.469 ± 0.0013	Above MRL
5	1.280	1.278	1.278	1.279 ± 0.0010	Above MRL
7	0.287	0.287	0.286	0.286 ± 0.0005	Below MRL
10	BDL	BDL	BDL		

MRL = Maximum Residue Limit as per Food Safety Standards Authority of India (FSSAI); BDL = Below Detectable Limits

Table 19: Residues of malathion in the tomato samples.

Interval (Days)	Residues (mg kg ⁻¹)				MRL (3 mg kg ⁻¹)
	R ₁	R ₂	R ₃	Mean ± SD	
0	8.488	8.488	8.419	8.465 ± 0.040	Above MRL
1	7.113	7.113	7.110	7.112 ± 0.002	Above MRL
3	5.911	5.876	5.945	5.911 ± 0.034	Above MRL
5	1.615	1.581	1.564	1.586 ± 0.026	Below MRL
7	BDL	BDL	BDL		
10	BDL	BDL	BDL		

MRL = Maximum Residue Limit as per Food Safety Standards Authority of India (FSSAI); BDL = Below Detectable Limits

SUMMARY AND CONCLUSIONS

Analysis of tomato fruit samples collected from the acephate and malathion treatments showed the residues up to 3 DAS and the samples collected after 5 DAS showed the residues below MRL (1, 3 mg kg⁻¹). Similarly, the samples collected from the chlorpyrifos treatment showed residues up to 5 DAS and thereafter the samples collected exhibited residues below MRL (0.5 mg kg⁻¹). The tomato fruit samples collected from acephate, chlorpyrifos and malathion treated fields showed below detectable levels of residues at 7, 10 and 7 DAS, respectively. Therefore, a safe waiting period of 5-7 days may be recommended for fruit picking after spraying with acephate, chlorpyrifos and malathion residues in tomato crop.

FUTURE SCOPE

The use of pesticides on vegetables is an inevitable part of agriculture but their unscientific usage may lead to serious health problems. Therefore this is the need of the hour to make some significant efforts to reduce the intake of pesticides for leading a healthier life. Research in this direction will allow bringing the pesticide residues below their maximum residue limits

and help to produce chemical-free, safe and good quality food for the upcoming generations.

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