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Determination of dimethoate, lambda-cyhalothrin and malathion residues in guava fruits using GCMS-tandem mass spectrometry

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ABSTRACT

Tephritidae fruit flies are considered key insect pest in guava which cause yield loss and degradation of quality. A trial was conducted in guava orchard during 2013 to study residue dissipation rate of two organophosphates, dimethoate and malathion and one synthetic pyrethroid insecticide, lambda-cyhalothrin. The insecticides were applied at recommended and double of the recommended dose on semi-ripe guava fruits. The fruits were randomly sampled at different time intervals, *i.e.* 0 (2 h), 3, 7 and 10 days after spray. After extraction and clean-up, the residues were estimated using GCMS tandem mass spectrometry. The half-life values at single and double dose applications were found to be 6.72 and 8.15 days for dimethoate, 4.41 and 4.71 days for lambda-cyhalothrin and 3 and 3.3 days for malathion. The residues reached below maximum residue limit (MRL) on zero day following recommended dose application of all insecticides. In double dose application, residues reached below MRL on zero day in malathion and lambda-cyhalothrin and on 3rd day in dimethoate. Washing of guava fruits removed 42.9 and 45.2% dimethoate, 33 and 36.8% lambda-cyhalothrin, 50 and 54.5% malathion residues at single and double dose applications in 0 day samples. Lambda-cyhalothrin was found most effective followed by dimethoate and malathion for controlling fruit fly in guava.

Key words: Dimethoate, GCMS-tandem mass spectrometry, lambda-cyhalothrin, malathion, residues.

INTRODUCTION

Guava is infested by several kinds of pests, but only few of them have been identified as pest of regular occurrence and causing serious damage. Tephritidae fruit fly is considered as a destructive group of insect that cause enormous economic losses in wide variety of vegetables, flowers and fruits especially in guava (Haseeb, 6). During summer, the fly spread quickly in guava orchards and produce edible rind and creamy white, yellow or pink flesh in fruits with sweet smell (Diamantidis *et al.*, 3). *Bactrocera invadens* and *B. dorsalis* have been reported as most abundant species of fruit fly causing about 92% infestation in guava (Jose *et al.*, 7). In some cases, where no control measures are adopted, the infestation becomes so serious that the entire guava crop gets damaged (Sarwar, 13). According to the World Health Organization (WHO, 20), food consumption consists 30% (based on mass) of fruit and vegetables and hence are considered as the most frequently consumed food group. Furthermore, because fruits and vegetables are mainly consumed raw or semi-processed, it is expected that they contain higher pesticide residues as compared to other food groups of plant origin, such as bread and other foodstuffs based on cereal processing (Claeys *et al.*, 1). Given the potential risk of pesticides for public health, the use of pesticides in fruits and vegetables production should be subjected to constant monitoring because pesticide residues make food commodities hazardous for consumption as well as pollute the environment (FAO/ WHO, 5). In addition, pesticide residues in exportable fruits and vegetables are not acceptable in the international market due to restrictions imposed by World Trade Organizatin (WTO, 21).

Keeping in view, this study was undertaken to determine the dissipation behaviour, residue half-life value and reduction of residues due to washing of guava fruits for three insecticides dimethoate, lambda-cyhalothrin and malathion, which are registered and most commonly used to control attack of fruit fly on guava grown under agro-climatic conditions of Hisar, India.

MATERIALS AND METHODS

Field experiment was conducted in an orchard of Department of Horticulture, CCS Haryana Agricultural University, Hisar India during summer (June-July) when temperature lies between 35 to 45°C. Two organo-phosphate insecticides, dimethoate (30 EC) and malathion (50 EC) and one synthetic pyrethroid insecticide, lambda-cyhalothrin (5 EC) was sprayed on the semi-ripe guava fruits at recommended (X) and double the recommended (2X) doses.

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Dimethoate was applied at 375 and 750 g a.i. ha^{-1}. Malathion was applied at 625 and 1250 g a.i. ha^{-1}, whereas lambda-cyhalothrin application rate was 45 and 90 g a.i. ha^{-1}. Each replicate of a treatment comprised of three adjacent trees. At each sampling-0 (2 h after spray), 3, 7 and 10 days of insecticides application, about 1 kg fruits were picked randomly from all the trees. The samples were transported to the laboratory in plastic bags and kept at -20°C in order to avoid any degradation of residues between sampling and analysis.

Residues from guava fruits were extracted according to the method proposed by Sharma (15). In required for analysis, the samples were cleaned up for removing the interfering substances by matrix solid phase dispersion extraction (MSPD) adopting the method reported by Lal *et al.* (11). A glass column (60 cm × 22 mm i.d.) was packed by placing cotton plug at the bottom, over which 1 cm layer of anhydrous sodium sulphate was laid. After tapping gently, adsorbent mixture consisting of thoroughly mixed Florisil: activated charcoal (3:0.3 w/w) was transferred to the column and tapped again to ensure compact packing. A 1 cm layer of anhydrous sodium sulfate was packed over the adsorbent mixture and the column was pre-washed with 50 ml of hexane. The concentrated extract was transferred to the column and eluted with 125 ml solution of hexane: acetone (9:1 v/v). Elutes were collected and concentrated to near dryness on rotary vacuum evaporator followed by gas manifold evaporator. Final volume was made to 2 ml in n-hexane for analysis by GCMS tandem mass spectrometry. In order to see the effect of washing in reducing the residues from guava fruits, the samples were washed under running tap water for 1 min. by gentle rubbing with hands after which the wash water was discarded. The washed guava fruits were kept on blotting paper for 30 min. to remove the excess water present over them. The samples were then processed for extraction and clean-up as per method published by Walter *et al.* (19).

For quantifying the residues of dimethoate, lambda-cyhalothrin and malathion, analysis was carried out using GCMS tandem mass spectrometry (Agilent 7890 A series with 7000 GCMS/ MS detector). The operating parameters were: injection port temperature: 280°C. Column: HP-5 (30 m × 0.32 mm i.d. × 0.25 µm film thickness). Oven temperature ramping was: 70°C (2 min. hold), increased at 25°C min^{-1} to 150°C (0 min. hold), increased at 15°C min^{-1} to 200°C (0 min. hold), increased at 8°C min^{-1} to 280°C (2 min. hold). Detector parameters were: source temperature, 230°C; emission current, 35 mA; quadrupole one (MS^1) temperature, 150°C; quadrupole two (MS^2) temperature, 150°C. Gas flow rates: Helium (carrier gas), 1 ml min^{-1} through column and 2.25 ml min^{-1} as collision flow/quench flow, nitrogen (collision cell), 1.15 ml min^{-1}. Spli ratio. 1:10; vacuum (high pressure), 2.23×10^{-5} torr; rough vacuum, 1.51×10^2 torr; injection volume, 2 ml. The retention time of dimethoate, lambda-cyhalothrin and malathion was observed to be 12.9, 23.6 and 15.7 min. respectively. The confirmation and quantification was achieved by developing a programming in Multiple Reactions Monitoring (MRM). Characteristic ions with relatively high in their intensity and strong anti-turbulence were selected as monitoring and quantitative ions.

RESULTS AND DISCUSSION

Standard stock solutions of dimethoate, malathion and lambda-cyhalothrin (1 µg ml^{-1}) were prepared in acetone. Linearity of calibration curve was studied using standard solutions. The response was found to be linear within dynamic range of 0.001 to 1 µg ml^{-1}. Limit of detection (LOD) and limit of quantification (LOQ) for dimethoate was 0.005 and 0.01 µg ml^{-1}, for malathion 0.001 and 0.005 µg ml^{-1} and for lambda-cyhalothrin was 0.0009 and 0.002 µg ml^{-1}, respectively. Recovery tests were performed for validation of analytical methodology by spiking guava fruits with dimethoate, lambda-cyhalothrin and malathion at 0.005 and 0.01 mg kg^{-1}. Average recoveries of dimethoate, lambda-cyhalothrin and malathion in guava fruits were to the extent of 80.7, 87.1 and 83.7%, respectively.

Application of dimethoate in field conditions resulted in initial deposits to the extent of 1.24 and 2.28 mg kg^{-1} at X and 2X application, respectively (Table 1). The residues dissipated gradually with time after application of both the doses. The dissipation of dimethoate on guava fruits followed first order kinetics with half-life ($t_{1/2}$) of 6.72 days in case of X and 8.15 days in case of 2X application. Residues of dimethoate reached below MRL value of 2 mg kg^{-1} on 0 day for X, whereas in 2X application, the residues (1.61 mg kg^{-1}) reached below MRL on 3rd day. Residues declined to 51.4% within a week for X dose, whereas for 2X application, 55.3% decline was observed in 10 days. Earlier, Szeto *et al.* (18) also observed more than 50% loss of dimethoate residues in asparagus within a week. In case of lambda-cyhalothrin, the initial deposits were 0.009 and 0.019 mg kg^{-1} for X and 2X doses, respectively. Thereafter, a gradual reduction in residues for both application doses was observed. The half-life value was found to be 4.41 for X and 4.71 days for 2X dose, respectively. The residues reached below MRL (0.03 mg kg^{-1}) on 0 day in both doses. Application of malathion on guava fruits resulted in initial deposit of 0.06 and 0.11 mg kg^{-1} for X and 2X

Table 1. Residues (mg kg^{-1})* of dimethoate, lambda-cyhalothrin and malathion in guava fruits.

Days after treatment	Dimethoate		Lambda–cyhalothrin		Malathion	
	X (375 g a.i. ha^{-1})	2X (750 g a.i. ha^{-1})	X (45 g a.i. ha^{-1})	2X (90 g a.i. ha^{-1})	X (625 g a.i. ha^{-1})	2X (1250 g a.i. ha^{-1})
0	1.24 ± 0.040	2.28 ± 0.233	0.009 ± 0.001	0.019 ± 0.001	0.06 ± 0.002	0.11 ± 0.002
3	0.83 ± 0.084 (33.1)	1.61 ± 0.150 (29.3)	0.005 ± 0.003 (44.4)	0.012 ± 0.002 (36.8)	0.03 ± 0.001 (50.5)	0.05 ± 0.001 (54.5)
7	0.602 ± 0.042 (51.4)	1.17 ± 0.281 (48.6)	0.004 ± 0.002 (55.6)	0.007 ± 0.004 (63.2)	0.01 ± 0.001 (83.3)	0.03 ± 0.003 (72.7)
10	0.535 ± 0.038 (56.8)	1.02 ± 0.130 (55.3)	0.003 ± 0.001 (66.7)	0.005 ± 0.002 (73.7)	BDL (100)	0.01 ± 0.001 (90.9)
Mean	0.801	1.52	0.005	0.010	0.025	0.047
Correlation coefficient		Correlation coefficient	Correlation coefficient	Correlation coefficient	Correlation coefficient	Correlation coefficient
r = - 0.9758		*r* = -0.9860	*r* = -0.9953	*r* = -0.9981	*r* = -0.9998	*r* = -0.9815
Regression equation		Regression equation	Regression equation	Regression equation	Regression equation	Regression equation
y = 3.0609-0.0362*x*		y = 3.3356-0.0349*x*	y = 0.9275-0.06395*x*	y = 1.2657-0.5805*x*	y = 1.7903-0.1115*x*	y= 2.0414-0.09741*x*
$t_{½}$ = 6.72 days		$t_{½}$ = 8.15 days	$t_{½}$ = 4.41 days	$t_{½}$ = 4.71 days	$t_{½}$ = 3 days	$t_{½}$ = 3.3 days

CD (p³0.05) for days = 0.001; for dose = 0.001; for days´dose = 0.002; for regression equation [residues (mg kg^{-1})´10^{3}] is taken
*Av. ± SD of three replicates
BDL below detectable level (0.50 mg kg^{-1}) of dimethoate, BDL below detectable level (0.001 mg kg^{-1}) of lambda–cyhalothrin, BDL below detectable level (0.01 mg kg^{-1}) of malathion, Maximum Residue Limit (MRL) of dimethoate, 2 mg kg^{-1} (Sharma, 2007), Maximum Residue Limit (MRL) of lambda-cyhalothrin, 0.03 mg kg^{-1} (*CODEX* Alimentarius), Maximum Residue Limit (MRL) of malathion, 4 mg kg^{-1} (Sharma, 2007)

application, respectively. More than 50% dissipation of residues was observed after both the applications on day 3. On 7th day 83.3 and 72.7% residues got dissipated in X and 2X application, respectively and reached below detectable level of 0.005 mg kg^{-1} on 10th day in X application. The dissipation of malathion in guava fruits followed first order kinetics with half-life ($t_{1/2}$) period of 3 and 3.3 days in both the doses, respectively. Therefore, it may be concluded that the residues of malathion, dimethoate and lambda-cyhalothrin at recommended dose application reached below MRL on 0 day. This information is important for determining the safe waiting period in guava. The results obtained in the present study regarding dimethoate residues are in agreement with those of Khan *et al.* (8) who reported initial deposit of 2.06 to 2.7 mg kg^{-1} residues in guava on zero day, which declined to 1.25 and 1.41 mg kg^{-1} on 3rd day after treatment. They reported that 87-94% residues dissipated over a period of 3 weeks. Mahdavian and Somashekar (12) reported 0.003-0.386 mg kg^{-1} of lambda-cyhalothrin residues in grapes.

Effect of washing after two hour of treatment of guava fruits (Table 2) resulted in reduction of residues of the three insecticides below MRL in both X and 2X applications. It was clear in 0 day samples from dimethoate treatment where residues were reduced from 1.24 to 0.707 mg kg^{-1} showing 42.9% decrease. In dimethoate, initial residual deposit of 2.28 mg kg^{-1} got reduced to 1.25 mg kg^{-1} in 0 day samples due to washing thereby showing 45.2% reduction; whereas on 7th day, only 12.9% reduction was observed. Washing of guava fruits removed 33% of lambda-cyhalothrin at X dose application from the initial residues of 0.009 mg kg^{-1}. These results revealed that percent reduction due to washing at X was more on 0 day (2 h after spray) samples than on subsequent days. As far as residue status of 2X application is concerned, initial deposit of 0.019 mg kg^{-1} on 0 day decreased to 0.012 mg kg^{-1}, thus showing 36.8% reduction of lambda-cyhalothrin residues. From X application of malathion on guava fruits the average initial residues of 0.06 mg kg^{-1} after two hour of application show 50 and 33.3% reduction due to washing in 0 and 3 day samples. At 2X application, the initial deposition of 0.11 mg kg^{-1} decreased to the 0.05 mg kg^{-1} thereby showing 54.5% reduction due to washing whereas on 3rd day residues reduced to 0.03 mg kg^{-1} thereby showing 40% reduction. Percent reduction of residues was found to be more in case of malathion and dimethoate in comparison to lambda-cyhaothrin mainly because of their higher water

Table 2. Effect of washing on the reduction of dimethoate, lambda-cyhalothrin and malathion residue (mg kg^{-1})*.

Days after treatment	Dimethoate				Lambda–cyhalothrin				Malathion			
	X (375 g a.i. ha^{-1})		2X (750 g a.i. ha^{-1})		X (45 g a.i. ha^{-1})		2X (90 g a.i. ha^{-1})		X (625 g a.i. ha^{-1})		2X (1250 g a.i. ha^{-1})	
	before washing	after washing	before washing	after washing	before washing	after washing	before washing	after washing	before washing	after washing	before washing	after washing
0	1.24	0.707 (42.9)	2.28	1.25 (45.2)	0.009	0.006 (33)	0.019	0.012 (36.8)	0.06	0.03 (50)	0.11	0.05 (54.5)
3	0.828	0.594 (28.3)	1.61	1.12 (30.6)	0.005	0.004 (20)	0.012	0.009 (25)	0.03	0.02 (33.3)	0.05	0.03 (40)
7	0.602	0.536 (10.9)	1.17	1.02 (12.9)	0.003	–	0.007	–	0.01	–	0.03	–
10	0.535	–	1.02	–								

*Av. ± SD of three replicates; Figures in parentheses is % reduction of residues

solubility. Lesser reduction of residues with passage of time was evident with all the three insecticides. It depends on their physical and chemical properties. Residues may get dried on the surface, adsorbed, bound to waxy material in greater proportion in the fruits and vegetables or translocated into the inner tissues of the plants, which enhance difficulty in removal of residues from fruits by washing with water (Sinha and Gopal, 16).

Dikshit *et al.* (4) observed that washing of 0 and 5 days brinjal fruit samples removed residues from 44 to 48% and 46 to 48%, in all the treatments at application of 12.5 to 75 g a.i. ha^{-1} of β-cyfluthrin and 35 g a.i. ha^{-1} of lambda-cyhalothrin, respectively. In a monitoring study conducted by and Kumari (9) and Kumari *et al.* (10) residues level of organo-chlorides, synthetic pyrethroids, organo-phosphate and carbamates were determined in three vegetables- brinjal, cauliflower and okra and it was observed that washing reduced the residues by 20-77%. Deen *et al.* (2) observed 35.6% reduction in residues of lambda-cyhalothrin from 0 day okra samples. Satpathy *et al.* (14) reported initial residues of malathion in cauliflower and tomato to be 0.22 and 0.19 mg kg^{-1}, which were removed to the extent of 39.0 and 41.0% after washing with tap water for one minute. Almost similar results were observed by Zafar *et al.* (22) where washing of okra fruits with tap water reduced lambda-cyhalothrin residues to 40-42.7% in 0 day and 30.8-33.9% in 5th day samples, respectively.

It was also inferred from the present study that dimethoate, lambda-cyhalothrin and malathion against fruit fly on guava exhibited variable efficacy in reducing oviposition marks/ fruit as well as maggot density over untreated check. The average number of oviposition marks/ fruit of guava varied from 5.5 to 7.25 before spray and 3.5 to 5.68 on 3 day after spray, while it was 7.87 in control. Lambda-cyhalothrin was found most effective with cumulative reduction of 73.07% in oviposition marks/ fruit after second spray followed by dimethoate (68.96%) and malathion (54.83%).

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