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Dissipation behavior and health risk assessment of deltamethrin in/on sapota under South Gujarat condition

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Abstract

The persistence and dissipation behavior of deltamethrin in/on sapota fruit under South Gujarat condition was investigated by using gas chromatography with electron capture detector (GC-ECD) after QuEChERS based extraction procedure. The linear dynamic range of deltamethrin in sapota fruit matrices was obtained between 0.01 to 0.1 µg/g. The recovery of deltamethrin in sapota fruit ranged from 98 to 101%. The relative standard deviation (RSD) ranged from 1.59% to 5.66%. The half-life of deltamethrin in sapota fruit were ranged from 1.74 and 2.19 days for recommended dose (28 g a.i./ha) and 1.83 and 2.00 days for double to recommended dose (56 g a.i./ha) for the year 2019-20 and 2020-21. The waiting period (WP) of deltamethrin ranges from 4.69 - 5.01 days under South Gujarat conditions. The dietary risk quotient (RQ) was found < 1 which signifies that sapota fruits collected from the field was safe for consumption of Indian population.

Keywords: Deltamethrin, dissipation, risk assessment, sapota, waiting period

Introduction

In Gujarat, sapota has attained the status of major fruit crop after mango, banana and pomegranate and now, Gujarat is the foremost sapota producing state in the country with 273.87 thousand MT production of total sapota which is cultivated on an area of 26.99 thousand ha with the productivity of 10.15 MT/ha (Anon., 2020) [1]. In particular to Gujarat region, about 16 insect pests and mites identified by scientists in last two decades from sapota orchards in Gujarat (Patel, 2002) [15] has raised to 23 insect and mite pests at present as per Bisane *et al.* (2018) [3]. Chemical-based control methods have quick, stable, and substantial effects with easy operation and low cost. These methods are therefore important for the control of sapota pests. At present 589 pesticide formulations registered for use in the India under the Insecticides Act, 1968 for the control of various types of pests (CBIRC, 2021) [4]. The extensive use of pesticides on one hand improved the agricultural productivity many folds, but on the other hand, they are posing a severe threat to the ecology and environment with widespread pollution. The present study is related to the persistence, dissipation and risk assessment of deltamethrin in/on sapota.

Deltamethrin (C₂₂H₁₉Br₂NO₃) (Fig. 1), is mobile and persistent in the environment, with greater toxicological effects. Therefore, conducting dietary risk assessments, of deltamethrin are assessed according to the Codex Alimentarius Commission (CAC) and current standards in the United States and European Union [7-9]. Sapota is an important fruit consumed either freshly or processed. Therefore, insecticide misuse for sapota has become an issue of global food safety and human health. However, no information is available on persistence and health risk management of deltamethrin on sapota fruit under South Gujarat condition. Therefore, two-year trial was conducted to generate data on persistence and dissipation kinetics of deltamethrin residue on/in sapota fruit.

Material and Methods

Materials and reagents

The NIST traceable chemical standard of deltamethrin (purity 99.62%) was procured from Sigma-Aldrich India limited. All the solvents used in the study were MS grade. Anhydrous magnesium sulfate was activated by heating at 400 °C in muffle furnace for 6 h before use and kept in desiccator.

All the glass wares were soaked in chromic acid solution and washed thoroughly with water. These were rinsed with acetone and air-dried before use. A technical grade of deltamethrin (10.75 mg) was accurately weighed on Oahu's (maximum capacity 210 g and sensitivity 0.001 g). The standards were then transferred to 25 ml capacity volumetric flasks. The deltamethrin content was initially dissolved with hexane: acetone (9:1 v/v) and final volume was also made up with hexane: acetone (9:1 v/v) which gave the concentration of deltamethrin 428.366 µg/ml. Primary standard of deltamethrin were diluted with hexane: acetone (9:1 v/v) to obtained intermediate standard of 40 µg/ml concentration which was further diluted in volumetric flask to prepare working standard of 0.01, 0.020, 0.04, 0.06, 0.08, 0.1 and 0.25 µg/ml concentrations. A commercial formulation Decis® 2.8% EC (Bayer crop Science Ltd, Mohali, Punjab) were purchased from the local market.

Thermo make Gas Chromatograph (Model, Trace GC-1310®) equipped with Electron Capture Detector (ECD) was used for the quantification of deltamethrin in samples. GC-ECD had DB-5MS, 30 m, 25 mm id, 0.25 µm FT Column, carrier gas helium, oven setting 170 °C/min (5 min.) to 220 °C /min (10 min.) to 280 °C/min (7 min.), total run time 54.0 min, retention time for deltamethrin (48.1 min); Column flow 1.0 ml/min, Injector PTV, injection mode split, split ratio 1:10, injection volume 1.0 µl, injection and detector temperature 250 °C and 300 °C, current 0.5 amp, makeup gas used nitrogen with flow of 25 ml/min. Data analysis was done by Chromaleon 7.2 software.

Field trial design

Field trial conducted at the Fruit Research Station, Gandevi, Navsari Agricultural University, Navsari during winter 2019-2020 and 2020-2021. This is geographically located at 20° 81' N latitude and 73° 02' E longitude at an altitude of 7.6 meters above the Mean Sea Level. Gandevi belongs to Agro-climate zone-I with Heavy rainfall area. The climate of the zone is characterized by fairly hot summer, moderately cool winter and warm monsoon. Annual average rainfall of this area is about 1400 to 1600 mm. The mean maximum temperature varies from 26° to 38 °C in summer and the minimum temperature ranges from 26° to 10 °C in winter. Insecticide formulations deltamethrin 2.8% EC trade name Decis® used during the field experiment. The test insecticide was sprayed twice onto the sapota fruits at intervals of 15 d at the recommended dose of 28 g a. i./ha and double to recommended dose 56 g a.i./ha. Sapota fruits sample collected after 2nd spray of deltamethrin at 0 day (2 hours after spray), 1, 3, 5 and 10 days.

Sample preparation

Treatment wise sapota fruit (2 kg) were collected from the experimental field and bring to the Food Quality Testing Laboratory, Navsari Agricultural University, Navsari for insecticide residue study. Each sample were analyzed as per the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method for fruits and vegetables (AOAC, 2007) [2] with certain modification. Chopped the sapota fruit sample (2 kg) and homogenized in high volume homogenizer. Weighed 15 g sample in 50 mL capacity polypropylene centrifuge tube. Add 15 ml of 1% acetic acid in acetonitrile (v/v) into the centrifuge tube and put at -20 °C for 20 minutes. Added 6.0 g anhydrous MgSO₄ and 1.5 g sodium acetate (anhydrous) shake well for 2 minutes and vortex for 30 seconds.

Centrifuged it for 3 minutes at 3500 rpm to separate organic layer. Transferred 6.0 supernatant organic layer into 15 ml untreated capacity centrifuge tube containing 300 mg PSA and 900 mg anhydrous MgSO₄ and vortex for 30 seconds. Centrifuged it for 2 minutes at 3500 rpm and take 2 ml supernatant (aliquot) in test tube and evaporated it to dryness with TurboVap at 40 °C. Finally make up the volume to 2 ml using Hexane: Acetone (9:1). Filtered it in to the glass vial and quantified on GC-ECD.

Method validation

Linear range, limit of detection (LOD), limit of quantification (LOQ), repeatability and recovery were determined for deltamethrin. The performance of the method was developed and validated as per SANTE guidelines (SANTE, 2019) [18]. The calibration curve for deltamethrin was obtained by plotting the peak area against the concentration of corresponding calibration standards. The LOD was determined by considering a signal-to-noise ratio of 3 with reference to the background noise obtained for the blank sample [response (height/area) of the detector vs. concentration]. To establish the linearity, multiple injections of sapota fruit matrix match standard viz., 0.01, 0.02, 0.04, 0.06, 0.08 and 0.1 µg/g of deltamethrin was performed and their response were recorded on GC-ECD. The Limit of quantification was determined by considering a signal-to-noise ratio of 10. The repeatability was expressed as a relative standard deviation (RSD), the recovery study in sapota fruit was performed at three spiking levels of deltamethrin i.e., 0.01, 0.06 and 0.10 µg/g respectively. The spiked samples were allowed to stand for 1 hr. before extraction to allow the spiked solution to penetrate the matrix and subsequently taken through the extraction and cleanup procedure. Matrix effect (ME) was evaluated by comparing the response obtained from solvent standards and matrix matched standards.

$$ME (\%) = \frac{(\text{Peak response of matrix standard} - \text{Peak response of solvent standard})}{\text{Peak response of matrix standard}} \times 100$$

Matrix effect was ignored when the ME value was in the range -20% to 20%, whereas the values > 20% indicated signal enhancement and value > -20%, indicated signal suppression (SANTE, 2019) [18]. The Per cent Recovery and Per cent Relative Standard Deviation (RSD) is the indicator of trueness and precision of any analytical method employed for the quantitative estimation of insecticides.

$$\text{Recovery (\%)} = \frac{\text{Recoverd value}}{\text{Fortifide level}} \times 100$$

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

Calculation of residue levels and risk assessment

Dissipation kinetics and Waiting period

The residue data was subjected to the first-order kinetics $C_t = C_0 e^{-kt}$ where C_t is the concentration after a lapse of time t ; C_0 is the initial concentration and k is the dissipation constant. The values of k were obtained from regression equation $y = a - bx$, where $y = \log C_t$, $a = \log C_0$, $b = k/2.303$, and $x = t$. The half-life (DT_{50}) and days to dissipation to 90% (DT_{90}) of deltamethrin were calculated from k values using the equation: $DT_{50} = \ln 2/k$ and $DT_{90} = \ln 10/k$, respectively. Waiting period of deltamethrin in sapota fruits was calculated by using formula $WP = (\ln C_0 - \ln MRL)/k$ (Mariappan and Kaithamalai,

2020) ^[11]. Where, C_0 is the apparent initial concentration (mg/kg), k is the dissipation rate constant and MRL is maximum residue limit of deltamethrin in sapota.

Health Risk Assessment

The deltamethrin residues obtained from sapota fruits at 0 day were used to work out the risk assessment. Dietary exposure and risk assessment calculations employed with the help of formula given below.

$$EDI = \frac{\text{Concentration of detected pesticide (mg/kg)} \times \text{food consumption (kg/day)}}{\text{Body weight of an adult (kg)}}$$

Body weight and sapota consumption (100 g/day) for different age group of Indian population was considered as per guideline of National Institute of Nutrition, Hyderabad (ICMR, 2011).

$$\text{Risk Quotient (RQ)} = \frac{\text{Estimated daily intake (EDI)}}{\text{Acceptable Daily Intake (ADI)}}$$

Where RQ is the risk quotient, EDI is the estimated daily intake (mg/kg, bw) and ADI is the acceptable daily intake (mg/kg, bw). The ADI values of deltamethrin is 0.01 mg/kg body weight (bw) (EFSA, 2020) ^[7]. Numerically, the RQ value more than 1 indicates that chemical poses risk to the consumers (Kansara *et al.*, 2021) ^[10].

Results and Discussion

Efficiency of method

The QuEChERS based extraction method used for analysis of deltamethrin residue in sapota fruit samples by GC-ECD and conformed by GC-MS. The linearity of the calibration curve was established in the range of 0.01-0.1 $\mu\text{g/g}$ with a correlation coefficient (R^2) of the calibration curve 0.998. The LOQ of this method was found to be 0.009 $\mu\text{g/g}$. while LOD being 0.003 $\mu\text{g/g}$. The attained method quantification limits are much lower than Codex MRL (0.01 $\mu\text{g/g}$), European Union maximum residue level (1 $\mu\text{g/g}$) and US MRL (1 $\mu\text{g/g}$) value for sapota fruit. The average recovery of deltamethrin was ranged from 98.56% to 101.41%, with relative standard deviation ranged from 1.59-5.66% when spiked the samples (0.01-0.1 $\mu\text{g/g}$ level of sampling) (Table 1). The acceptable range of recoveries for trace residue analysis has been fixed between 70% and 120% (SANTE, 2019) ^[18] with RSD of <20% (European Commission, 2010) ^[8]. Thus, the method optimized in the present study provided satisfactory level of precision and accuracy.

Persistence and dissipation of deltamethrin in sapota fruit

The dissipation behavior of deltamethrin pertaining to recommended (28 g a i/ha) and double to recommended dose (56 g a.i./ha) in sapota fruit from the open field experiment are presented in Table 2. The initial deposits of deltamethrin residues in sapota fruits during year 2019-20 and 2020-21 were $0.049 \pm 0.011 \mu\text{g/g}$ and $0.065 \pm 0.020 \mu\text{g/g}$, respectively. In RD residues of deltamethrin were dissipated up to 62.38 per cent and 75.38 per cent within the 3 days during year 2019-20 and 2020-21, respectively. In first order kinetics, during the year 2019-20 and 2020-21, the coefficient of determination (R^2) of deltamethrin in RD were 0.973 and 0.924, respectively. The half-life (DT_{50}) was 2.19 days and

1.74 days and DT_{90} was 7.26 days and 5.77 days, respectively during 2019-20 and 2020-21. The present finding on DT_{50} of deltamethrin @ recommended dose is matched with the results reported with DT_{50} for cabbage 1.34 days (Reddy and Reddy, 2011) ^[17], bittergourd 1.9 days (Gupta *et al.*, 2012) ^[5], chili at four agro-climatic zones (AAU, Anand, ANGRAU, Hyderabad, BCKV, West Bengal and PAU, Ludhiana) was found to be 0.69, 0.61, 1.19 and 0.36 days, respectively (Pandher *et al.*, 2012) ^[13], green tea leaves 3.04 days (Paramasivum and Chandrasekaran, 2014) ^[14], cabbage 1.12 days (Gupta *et al.*, 2015) ^[6], tomato and eggplant 4.80 and 1.77 days (Mukherjee *et al.*, 2015) ^[12], brinjal fruits 1.76 days (Patel *et al.*, 2016) ^[16], tea leaves grown at Kamalapur, Teok and Assam Agricultural University, Assam 1.78 days (Sharma *et al.*, 2017) ^[19] and cucumber ranged from 2.5 to 4.9 days (Sharma *et al.*, 2018) ^[20] shows more or less similar dissipation pattern of deltamethrin in different crops under different agro-climatic conditions.

The deltamethrin residues in treatment receiving double to recommended dose (2 x RD) in sapota fruits during 2019-20 and 2020-21 were presented in Table 2. The highest deltamethrin residues $0.069 \pm 0.016 \mu\text{g/g}$ and $0.099 \pm 0.032 \mu\text{g/g}$ were found at 0 day (2 hrs. after application) and continued to dissipated up to 65.92 per cent and 68.87 per cent within the 3 days during the year 2019-20 and 2020-21, respectively. In first order kinetics, the coefficient of determination (R^2) of deltamethrin in 2 x RD were 0.968 and 0.979 respectively, While, in case of half-life (DT_{50}) were 2.00 days and 1.83 days and DT_{90} were 6.64 days and 6.08 days, respectively, during 2019-20 and 2020-21.

The present finding on DT_{50} of deltamethrin at 2xRD is differing from the results reported with DT_{50} for bittergourd 2.1 days (Gupta *et al.*, 2012) ^[5], chili at four Agro-climatic zones (AAU, Anand, ANGRAU, Hyderabad, BCKV, West Bengal and PAU, Ludhiana) was found to be 1.14, 0.70, 2.06 and 0.38 days, respectively (Pandher *et al.*, 2012) ^[13], green tea leaves 3.54 days (Paramasivum and Chandrasekaran, 2014) ^[14], cabbage 0.91 days (Gupta *et al.*, 2015), tomato and eggplant 4.79 and 2.66 days (Mukherjee *et al.*, 2015) ^[12], brinjal fruits 2.10 days (Patel *et al.*, 2016) ^[16], tea leaves grown at kamalapur, Teok and Assam Agricultural University, Assam 1.94 days (Sharma *et al.*, 2017) ^[19] and cucumber ranged from 2.5 to 4.9 days (Sharma *et al.*, 2018) ^[20]. This variation might be the resultant of the prevailing environmental factors and processes viz., temperature, relative humidity, volatilization and photo-degradation at field conditions (Gupta *et al.*, 2012; Pandher *et al.*, 2012; Gupta *et al.*, 2015; Sharma *et al.*, 2017; Sharma *et al.*, 2018) ^[5, 13, 6, 19] which are not consistent in different geographical regions.

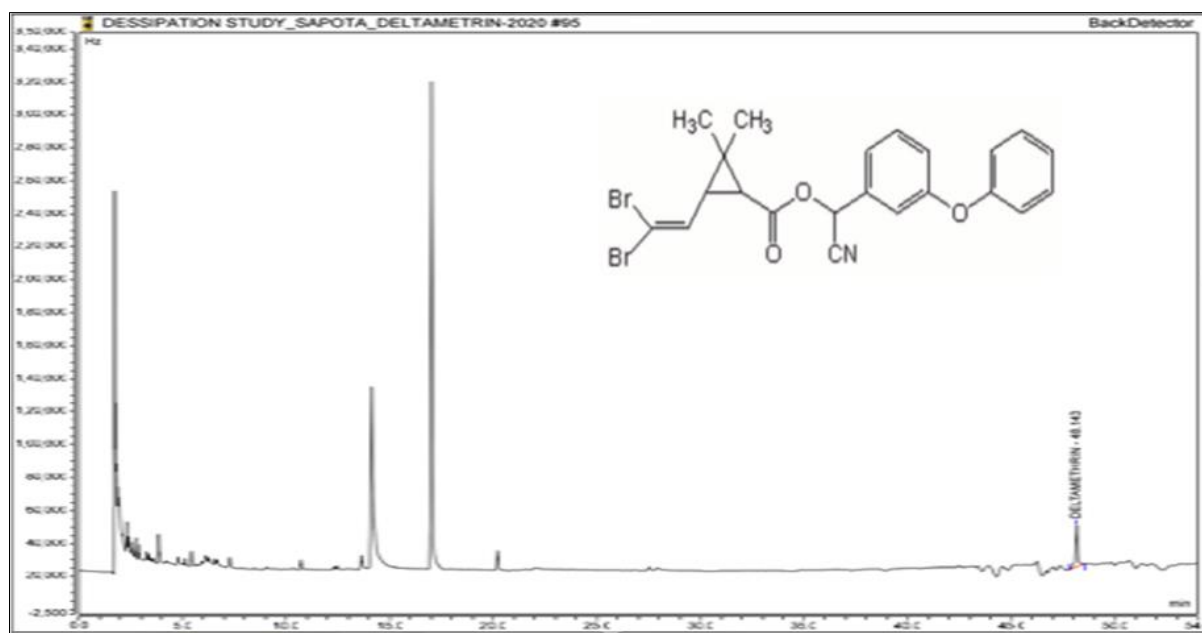
Health risk assessment

The risk quotient (RQ) on 0 days (after 2 hrs.) due to application of RD of deltamethrin were ranges from 0.0082-0.0380 and 0.0108-0.0504 respectively, during year 2019-20 and 2020-21. However, in case of 2 x RD, RQ ranges from 0.0115-0.0535 and 0.0165-0.0767 respectively, for the year 2019-20 and 2020-21 (Table 3). The dietary risk quotient (RQ) was found < 1 at 0-day (2 hr) deltamethrin residues in RD and 2 x RD which indicates that sapota fruits were safe for consumption of Indian population.

Table 1: Method validation studies of deltamethrin in sapota fruits

Parameter	Particular		Deltamethrin
Linearity (n=5)	Calibration range		0.01 to 0.1(µg/g)
	Regression equation		$y = 14195x + 445.19$
	$R^2 \{R^2 \geq 0.99\}$		0.998
	Matrix effect {-20% - 20%}		1.31 to 4.45%
Sensitivity (n=5)	LOD (µg/g)		0.003
	LOQ (µg/g)		0.009
Accuracy (n=7)	% Recovery {70-120}	F level (µg/g) 0.01 to 0.10	98.56% to 101.41%
Precision (n=7)	%RSD {≤ 20%}	F level (µg/g) 0.01 to 0.10	1.59 to 5.66%

N: number of replication; R²: Correlation coefficient; LOD: Limit of detection; LOQ: Limit of quantification; F level: Fortification level, RSD: Relative standard deviation; value given in parenthesis { } are the standard acceptance criteria as per SANTE 2019.

**Fig 1:** Chromatogram of matrix match standard of deltamethrin on GC-ECD**Table 2:** Persistence and dissipation behaviour of deltamethrin in sapota fruit year and

DAA	2019-20				2020-21			
	Dose (RD @ 28 g a.i./ha)		Dose (2XRD @ 56 g a.i./ha)		Dose (RD @ 28 g a.i./ha)		Dose (2XRD @ 56 g a.i./ha)	
	Residue (µg/g ±SD)	Dissipation (%)	Residue (µg/g ±SD)	Dissipation (%)	Residue (µg/g ±SD)	Dissipation (%)	Residue (µg/g ±SD)	Dissipation (%)
0	0.049±0.011	0	0.069 ±0.016	0	0.065 ±0.020	0.00	0.099±0.032	0.00
1	0.031±0.006	36.58	0.041 ±0.009	40.30	0.032 ±0.015	50.77	0.059±0.020	40.69
3	0.018±0.007	62.38	0.024 ±0.006	65.92	0.016 ±0.010	75.38	0.031±0.008	68.87
5	BQL	-	BQL	-	BQL	-	BQL	-
Reg. eq.	$y = 0.0464e^{-0.317x}$		$y = 0.0645e^{-0.347x}$		$y = 0.0574e^{-0.399x}$		$y = 0.0934e^{-0.379x}$	
R ²	0.973		0.968		0.924		0.979	
DT ₅₀	2.19 days		2.00 days		1.74 days		1.83 days	
DT ₉₀	7.26 days		6.64 days		5.77 days		6.08 days	
WP	Codex	5.01 days	Codex	5.57 days	Codex	4.69 days	Codex	6.05 days
	EU	1 day	EU	1 day	EU	1 day	EU	1 day
	US	1 day	US	1 day	US	1 day	US	1 day

Table 3: Health risk assessment in sapota for deltamethrin

Group	Age group (year)	Body weight (kg)	2019-20				2020-21			
			RD		2 x RD		RD		2 x RD	
			EDI	RQ	EDI	RQ	EDI	RQ	EDI	RQ
Children	1-3	12.9	0.0004	0.0380	0.0005	0.0535	0.0005	0.0504	0.0008	0.0767
	4-6	18	0.0003	0.0272	0.0004	0.0383	0.0004	0.0361	0.0006	0.0550
	7-9	25.1	0.0002	0.0195	0.0003	0.0275	0.0003	0.0259	0.0004	0.0394
Boy	10-12	34.3	0.0001	0.0143	0.0002	0.0201	0.0002	0.0190	0.0003	0.0289
Girl	10-12	35	0.0001	0.0140	0.0002	0.0197	0.0002	0.0186	0.0003	0.0283
Boy	13-15	47.6	0.0001	0.0103	0.0001	0.0145	0.0001	0.0137	0.0002	0.0208

Girl	13-15	46.6	0.0001	0.0105	0.0001	0.0148	0.0001	0.0139	0.0002	0.0212
Boy	16-18	55.4	0.0001	0.0088	0.0001	0.0125	0.0001	0.0117	0.0002	0.0179
Girl	16-18	52.1	0.0001	0.0094	0.0001	0.0132	0.0001	0.0125	0.0002	0.0190
Man	Sedentary work	60	0.0001	0.0082	0.0001	0.0115	0.0001	0.0108	0.0002	0.0165
	Moderate work	60	0.0001	0.0082	0.0001	0.0115	0.0001	0.0108	0.0002	0.0165
	Heavy work	60	0.0001	0.0082	0.0001	0.0115	0.0001	0.0108	0.0002	0.0165
Woman	Sedentary work	55	0.0001	0.0089	0.0001	0.0125	0.0001	0.0118	0.0002	0.0180
	Moderate work	55	0.0001	0.0089	0.0001	0.0125	0.0001	0.0118	0.0002	0.0180
	Heavy work	55	0.0001	0.0089	0.0001	0.0125	0.0001	0.0118	0.0002	0.0180

Conclusion

QuEChERS method adopted for extraction with acetonitrile and quantification on GC-ECD (deltamethrin) is accurate, precise and sensitive enough as per SANTE guidelines (2019)^[18] for estimation of deltamethrin from sapota fruit. Deltamethrin followed first order dissipation kinetics (R^2) in sapota fruit with the half-life (DT_{50}) ranges from 1.74 - 2.19 days, 90 per cent dissipation time (DT_{90}) ranges from 5.77 - 7.26 days. The waiting period of deltamethrin ranges from 4.69 - 5.01 days in RD under South Gujarat conditions. The dietary risk quotient (RQ) was found < 1 which signifies that sapota fruits collected from the field was safe for consumption of Indian population

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