Original Research Article

Dissipation dynamics and risk assessment of imidacloprid in grape berries using LC- MS for food safety

ABSTRACT

Aim: The present study was taken up to assess the imidacloprid residue pattern in order to define strategies for improvement of quality and safetymainly healthfulness in grapes.

Place and duration of study: The present study was carried out in Tamil Nadu during the period 2020-2021.

Methodology: Dissipation studies were carried out in a farmer's field at Theni, India (9^{o} N latitude and 76^{o} E longitude and 375m above mean sea level) with all good agricultural practices**.** During this study Grapes (Muscat Hamburg variety) was grown and the commercial formulation of imidacloprid 17.8% SL was applied at recommended dose (53 a.i ha^{-1}) and double dose (106 a.i ha^{-1}) Two sprays were given with an interval of 10 day(at which stage first application was given)by using a power operated sprayer as 500 L ha^{-1} spray fluid for dissipation study. The samples were processed by adopting modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method

Results: The initial deposit imidacloprid in grape berries was 0.65 and 1.56 $^{mg\ kg\text{-}1}$ at the recommended dose (53 g a.i ha^{-1}) and double dose respectively (106 g a.i ha^{-1}). The insecticide exhibited a slower dissipation rate (Table 3) and reached below its quantification level (<0.01 $^{mg\ kg\text{-}1}$) in grape berries on 25^{th}, 30^{th} day at recommended and double doses respectively.

Conclusion: The toxicological dietary risk assessment data shows that risk quotient is less than one which confirms safer to consumers. Pre-harvest interval does not apply for imidacloprid at recommended dose as being grapes harvested on the day of application itself were found safe for consumption and PHI/ safe waiting period of 2 days is suggested for double dose for harvesting.

Keywords: Grapes, Imidacloprid, method validation, dissipation, risk assessment, food safety

1. INTRODUCTION

Grapes (Vitis vinifera L.) are an extremely significant non-climacteric fruit that have adapted to the humid tropical and sub-tropical climates found in the Indian

subcontinent. One of the fruits with the highest carbohydrate content (15–18 g per 100 g), high calorie content (65 kcal), and low glycemic index (GI), grape berries are popular both as fresh and processed foods (in the range of 43-59).Commercial grape growing often brings unwanted pests to the vineyards. Vines in India are attacked by up to 60 different types of insects and a few mites(1). Because of high incidence and damage from these pests, grape growers often use higher application rate of the insecticides with multiple sprayings at different crop growth stages, even up to harvest. Evidence showed that approximately 7 per cent of pesticides utilized in agriculture were applied in grapes (2). In most areas of Tamil Nadu (India), the grape crop is grown by implying a higher usage of chemical inputs (pesticides) without any knowledge on optimal safe usage. The growth dilution effect allows pesticides given to fruit while it is still developing to break down more quickly (3). In contrast, when administered later in the fruit's development, they have a greater chance of being absorbed by the fruit.Increased awareness in the public about pesticide residues in fruits and vegetables, as well as the potential health impacts, necessitates the use of effective pesticide residue removal procedures in the home.

Imidacloprid is one the highly used chemical formulation against thrips and it registered under (CIB&RC, 2021). Imidacloprid is having water solubility (610 mg L^{-1} at 20^{o}C) melting point (143^{o}C), Log K_{ow} (0.57) and vapour pressure (2×10^{-4} at 20^{o}C) (4). More over studies on the residue dynamics of pesticides in grapes are very less under Indian conditions and no studies from Tamil Nadu. Considering these conditions, the present study was taken up to assess the imidacloprid residue pattern in order to define strategies for improvement of quality in grapes and mainly healthfulness in grapes. Photodegradation of imidacloprid produces 6-chloronicotinic acid, which is counted as part of the total residue. Because of the increased concern for the safety of certain pollinators, the metabolite 6-CNA has been studied alongside the parent chemical.

2. material and methods

2.1. Chemical and reagents

The analytical standards for imidacloprid (98.3 %) and 6-chloronicotinic acid (98.9 %) were acquired from Sigma Aldrich in Bangalore, India. The HPLC grade ethyl acetate (99.7%), acetonitrile (97%), and hexane (95%) were obtained from Sisco Research Laboratories in Mumbai, India.Himedia Laboratories, Mumbai, India, supplied analytical quality sodium chloride and anhydrous magnesium sulphate, both of which were obtained from Merck, Mumbai, India, and both of which were purified to greater than 99%.Agilent Technologies, USA supplied the primary secondary amine (PSA, 40 m) and graphitized carbon black (GCB). Fisher Scientific Limited provided formic acid (> 99.9% purity) (Czech Republic).The commercial formulation of imidacloprid 17.8% SL (TATAMIDA) was purchased locally in pesticide dealer shop at Coimbatore, Tamil Nadu, India.

2.2 Preparation of standard solutions

In order to prepare the imidacloprid stock solution (400 mg/L) in HPLC-grade acetonitrile, about 10.18 mg of the analytical standard was weighed into a calibrated Glass A volumetric flask (25 mL).The secondary stock solution (40 mg/L) was prepared from the stock solution in 25 mL volume by transferring 2.5 mL. TCalibration and spiking standards were prepared by serially diluting the secondary stock solution to concentrations between 0.01 and 0.1 mg/L.All the standard solutions were stored in deep freezer at -20^{o}C until use. Grapes of varying maturity were used to make the matrix-matched standard solutions of 0.01, 0.025, 0.05, 0.075, and 0.1 mg/L.

2.3. Methods

2.3.1. Field experiment details

A farmer's field in Theni, India (9^{o} N latitude, 76^{o} E longitude, and 375m above mean sea level) was used for a dissipation experiment using standard farming practices.Treatments consisted of three sets of 50m2 plots that had never been sprayed with imidacloprid before the trial began.In this experiment, grapes (Muscat Hamburg variety) were cultivated, and the Central Insecticide Board and Registration Committee (5) recommended dose (53 a.i ha-1) and double dose (106 a.i ha-1) of the commercial formulation of imidacloprid 17.8% SL were applied 45 days after flowering, when grape berries begin to form.Sprays were applied with a 10-day interval using a power-operated sprayer and 500 litres of spray fluid per hectare for the dissipation study.During field trial, a total rainfall of 116 mm was received with 28.16^{o}C and 19.16^{o}C as maximum, minimum temperatures respectively. A relative humidity of 78.16% was recorded for the entire trial period.

2.3.2. Sample collection and preparation

Random samples of grapes were taken from each replication immediately following spraying (0 hours), 1, 3, 5, 7, 10, 15, 20, 25, 30, and 35 days, and then brought to the lab for analysis.Samples were homogenised by using a high-volume blade homogeniser and stored at -40^{o}C temperature. For decontamination study samples were treated with simple household methods.

2.3.3.Extraction and clean-up for dissipation studies

The samples were processed using a derivative of the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) technique (6). After transferring 10 g of the material to a 50 ml centrifuge tube, 10 ml of acetonitrile was added, and the mixture was agitated with a vortexer for one minute.The mixture was then centrifuged at 6000 rpm for 10 minutes after being mixed well by vortexer and adding another 4 g of anhydrous MgSO4 and 1 g of NaCl.A 6 mL aliquot of the supernatant was centrifuged, and the contents of the tube were added to 15 mL of centrifuge tube containing 100 mg of Primary

Secondary Amine (PSA), 600 mg of anhydrous magnesium sulphate (MgSO4), and 10 mg of graphitized carbon black (GCB).The mixture was centrifuged at 3000 rpm for 10 minutes after being vortexed for 30 seconds.Finally, a 1 mL sample was obtained, filtered through a 0.2 m membrane syringe filter, and placed in a 1.0 mL LCMS autosampler vial for analysis of imidacloprid and 6-chlornicotinic acid residues.

2.3.4. LC-MS instrument

The residues were detected and estimated using a Shimadzu 2020 series LCMS fitted with an SPD-M20A (Diode array detector) and a reverse phase C18 (Eclipse plus-Agilent) column measuring 250mm in length, 4.6 mm in internal diameter, and 5 in particle size and stored in a 40^{0}C column oven.To get an accurate reading, we mixed 50 millilitres of LiChrosolv grade acetonitrile in 1 millilitre of ultra-pure water with 0.1% formic acid.In order to discharge the mobile phase at the aforementioned ratio while maintaining a constant flow rate of 0.4 mL/min, the LCMS pump was run in binary mode at 55 kgf/cm2 pressure.Under the conditions already described, the wavelength and retention time at which imidacloprid residues could be identified was 7.74.The chromatograms and the subsequent calculations based on the peak regions obtained were analysed using Shimadzu lab solutions software.The recovery, reproducibility, linearity, detection limit, quantification limit, sensitivity, trueness, precision, and matrix effect of the method employed to identify imidacloprid residues in grape matrices were evaluated in accordance with SANTE recommendations (7).The repeatability of the recovery investigation was determined by calculating the relative standard deviation of individual recoveries at five different fortification levels (0.01, 0.025, 0.05, 0.075, and 0.1 mg kg^{-1}).After generating the calibration curve using imidacloprid standard solutions in concentrations ranging from 0.005 mg kg-1 to 0.1 mg kg-1, the linearity was examined and validated.In order to evaluate the sensitivity of the newly created approach, the method's limit of detection was determined by spiking the imidacloprid with various matrices at the lowest concentration level possible while still satisfying the requirements of the analytical method.For each spiking level of 0.01, 0.025, 0.05, 0.075, and 0.1 mg kg^{-1} of both matrices, the proposed methodology was evaluated in terms of repeatability (Relative Standard Deviation).Comparison of the response to solvent standards and matrix-matched standards was used to calculate the matrix effect (ME) and estimated using (8)

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

2.3.5. Statistical analysis

The imidacloprid residue was calculated by using (9)

$$\text{Residue } (^{\text{mg kg-1}}) = \frac{\text{A1} \times C \times I1 \times F}{\text{A2} \times W \times I2}$$

Where, A_1 = Peak area of the sample, A_2 = Peak area of the standard, I_1 = Injected volume of standard (µL), I_2 = Injected volume of sample (µL), C = Concentration of standard solution (mg/L), F = Final volume of the sample (mL) and W= weight of the sample (kg). The imidacloprid residue data thus obtained from the field experiments were subjected to first-order dissipation kinetics equation $Ct = Coe^{-kt}$, where, Ct is the pesticide concentration ($^{mg\ kg-1}$) at time t (day), C_o is the apparent initial concentration ($^{mg\ kg-1}$), k is the dissipation rate constant (10). The half-life of imidacloprid was determined as DT_{50} = ln2/k (11).

The maximum residue limit (MRL) has been published by European pesticide database for imidacloprid in grapes was 1.0 $^{mg\ kg-1}$ (12) and pre-harvest interval (PHI) of imidacloprid was calculated using$PHI = [InC_0=InMRL]/k$

2.3.6. Dietary risk assessment

By calculating the maximum residue concentration (mg kg-1) with the food consumption rate (kg/day) divided by the mean body weight of an adult, the estimated daily intake (EDI) of imidacloprid residue was obtained.The risk quotient (RQ) was determined by dividing the estimated daily intake (EDI) by the applicable acceptable daily intake (ADI) expressed in mg kg-1 body weight (bw) per day in order to quantify the long-term risks associated with pesticide ingestion in comparison to toxicological data.Imidacloprid's acceptable daily intake (ADI) is 0.06 milligrammes per kilogramme of body weight per day(13). An Indian adult is thought to weigh 55 kilogrammes (14) on average, and they should eat 300 grammes (15) of vegetables every day.When RQ is less than 1, we know that long-term human dietary intake of imidacloprid has no unacceptable danger, and when it's greater than 1, we know that the risk is too high(Table.7).

3. RESULTS AND DISCUSSION

Recovery percentage, Relative Standard Deviation (RSD), and linearity were calculated to validate the analytical method used to quantify the residue of imidacloprid and its metabolite 6-chloronicotinic acid in both immature and mature grape berries.Recovery rates for imidacloprid and its metabolite 6-chloronicotinic acid in grapes were all within the acceptable range of 70-120%, as recommended by the SANTE guidelines.(Table 1, 2,3 &4) and less than20% for RSD (7). For both matrices, we found that the linearity of the approach was between 0.005 and 0.1 mg/L(Table. 5).The linear regression equation for imidacloprid and 6-chloronicotinic acid standards was y = 1E + 06x + 680.41 and y = 3E + 06x + 9393.95, respectively.The LOD and LOQ were 0.005 and 0.01 mg kg^{-1} respectively. The method's LOQ (0.01 mg kg-1) was below EU Pesticide database's MRL (1.0 mg kg-1). Analytical technique RSDs ranged from 0.17 to 12.33.

3.1. Dissipation of imidacloprid residues in grape berries

When applied at the prescribed dose (53 g a.i ha^{-1}), the initial deposit of imidacloprid in grape berries was 0.65 mg kg^{-1}, and when applied at double the

permissible dose, the initial deposit was 1.56 mg kg^{-1} (106 g a.i ha^{-1}).The insecticide broke down more slowly (Fig.1 and Table 6) and was below its quantification level (0.01 mg kg-1) in grape berries on the 25th and 30th day at the recommended dose and the double dose and This was because imidacloprid works in a systemic way in plants, so residues move quickly into the insides of berries(16). The present findings are more or less similar to results of studies in grapes where imidacloprid residues reached to BDL with half-lives of 2.21 and 2.94 days (17) and 2.35 and 2.97 days (18). A study was conducted with spirotetramat + imidacloprid where imidacloprid was degraded by 10^{th} day to BDL with half live of 5.07 days (19). However, higher half-life (16.6 days) was reported for imidacloprid in grapes (20) due to variation in insecticide formulation, dosage and environmental factors. The present findings are more or less in agreement with studies other than grapes where imidacloprid was dissipated to BDL on 15^{th} day in sweet orange (21), 10^{th} day in kinnow mandarin (22), 10^{th} day in pomegranate (23).

Table 1. Recovery percentage of imidacloprid residues in immature grapes

Spiked level (µg g^{-1})	Recovery (%)							Mean recovery (%) * ± SD	RSD (%)
	R1	R2	R3	R4	R5	R6	R7		
0.01	106.09	115.45	117.24	112.38	91.80	101.24	106.18	107.19 ± 8.86	8.26
0.025	84.80	86.69	80.00	87.82	87.71	94.46	94.13	87.94 ± 5.09	5.78
0.05	85.90	86.70	90.80	91.34	93.12	91.90	94.17	90.64 ± 3.24	3.57
0.075	101.34	97.57	101.90	99.76	98.63	94.91	103.36	99.64 ± 2.87	2.88
0.1	102.27	102.50	104.17	100.74	105.37	102.50	104.17	103.10 ± 1.55	1.50

***Mean of seven replications, SD- Standard Deviation, RSD- Relative Standard Deviation**

Table 2. Recovery percentage of imidacloprid residues in mature grapes

Spiked level (µg g^{-1})	Recovery (%)							Mean recovery (%) * ± SD	RSD (%)
	R1	R2	R3	R4	R5	R6	R7		
0.01	100.41	109.73	116.17	102.10	105.90	112.05	104.68	107.29 ± 5.63	5.25
0.025	105.11	117.40	101.42	109.45	101.47	100.68	110.95	106.64 ± 6.25	5.86

0.05	86.98	88.08	88.64	94.64	88.81	89.53	91.39	89.72 ± 2.56	2.85
0.075	109.12	104.64	102.31	91.45	104.93	87.45	97.75	99.66 ± 7.84	7.87
0.1	90.45	91.44	85.51	95.00	91.44	85.51	95.00	90.62 ± 3.91	4.32

*Mean of seven replications, SD- Standard Deviation, RSD- Relative Standard Deviation

Table 3. Recovery percentage of 6-chloronicotinic acid residues in immature grapes

Spiked level ($\mu g\ g^{-1}$)	**Recovery (%)**							**Mean recovery (%) * ± SD**	**RSD (%)**
	R1	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**		
0.01	84.06	94.58	90.21	97.02	81.48	87.50	75.93	87.25 ± 7.41	8.50
0.025	98.92	108.89	107.22	103.17	95.40	104.05	95.26	101.84 ± 5.46	5.36
0.05	113.32	110.58	106.42	101.68	112.47	109.01	112.02	109.36 ± 4.12	3.77
0.075	116.55	114.83	119.33	119.20	119.12	110.78	111.86	115.95 ± 3.58	3.09
0.1	114.46	113.51	116.76	111.45	116.49	116.76	111.45	114.41 ± 2.37	2.07

*Mean of seven replicates, SD- Standard Deviation, RSD- Relative Standard Deviation

Table 4. Recovery percentage of 6-chloronicotinic acid residues in mature grapes

Spiked level ($\mu g\ g^{-1}$)	Recovery (%)							Mean recovery (%) * ± SD	RSD (%)
	R1	R2	R3	R4	R5	R6	R7		
0.01	78.76	92.88	89.27	79.10	76.50	94.92	87.57	85.57 ± 7.41	8.66
0.025	83.48	85.60	72.23	75.71	78.64	81.90	73.58	78.73 ± 5.13	6.51

0.05	96.72	94.33	88.45	94.46	88.38	92.84	93.60	92.68 ± 3.15	3.40
0.075	92.22	86.42	85.02	90.62	91.22	89.13	83.28	88.27 ± 3.40	3.85
0.1	88.28	90.37	92.25	96.29	89.45	91.04	93.24	91.56 ± 2.66	2.91

***Mean of seven replicates, SD- Standard Deviation, RSD- Relative Standard Deviation**

Table 5. Linearity parameters and matrix effect for imidacloprid and 6-CNA residues in different grape matrices

Pesticides	Matrix	Calibration range (mg/L)	Regression equation	Correlation coefficient (R^2)	Matrix effect (%)
Imidacloprid	Solvent	0.005-0.1	y = 1E+06x + 5254.1	0.9999	-
	Immature grapes	0.005-0.1	y = 2E+06x - 2285.5	0.9921	4.32
	Mature grapes	0.005-0.1	y = 840790x - 773.9	0.9959	5.61
	Juice	0.005-0.1	y = 3E+06x + 2285.8	0.9998	5.09
	Squash	0.005-0.1	y = 3E+06x + 125.78	0.9997	6.17
	Raisin	0.005-0.1	y = 4E+06x - 232.39	0.9997	6.40
6-chloroniconitic acid	Solvent	0.005-0.1	y = 3E+06x - 3815.8	0.9998	-
	Immature grapes	0.005-0.1	y = 148936x - 97.683	0.9996	5.44
	Mature grapes	0.005-0.1	y = 83366x - 172.24	0.9967	2.56
	Juice	0.005-0.1	y = 4E+06x + 923.27	0.9999	4.40
	Squash	0.005-0.1	y = 5E+06x - 1525.9	0.9999	5.16
	Raisin	0.005-0.1	y = 3E+06x - 421.39	0.9999	3.67

Table 6. Persistence and dissipation of imidacloprid 17.8 % SL residues in/on grapes (mg kg^{-1})

Residues (mg kg^{-1})												
Imidacloprid @ 53 g a.i ha^{-1}							Imidacloprid @ 106 g a.i ha^{-1}					
Days after treatment	R1	R2	R3	Mean± SD	RSD (%)	Dissipation (%)	R1	R2	R3	Mean ± S D	RSD (%)	Dissipation (%)
0 (2hrs)	0.65	0.72	0.68	0.68 ± 0.03	4.98	-	1.60	1.52	1.56	1.56 ± 0.04	2.37	-
1	0.44	0.47	0.50	0.47 ± 0.03	5.99	30.88	1.08	1.06	1.06	1.07 ± 0.01	1.33	31.41
3	0.46	0.41	0.42	0.43 ± 0.02	5.51	36.76	0.74	0.74	0.73	0.74 ± 0.01	1.14	52.56
5	0.36	0.33	0.41	0.37 ± 0.04	11.11	45.58	0.71	0.69	0.69	0.70 ± 0.01	1.80	55.12
7	0.24	0.23	0.19	0.22 ± 0.03	13.36	67.64	0.44	0.42	0.42	0.43 ± 0.01	2.90	72.43

10	0.11	0.10	0.12	0.11 ± 0.01	7.94	83.82	0.24	0.26	0.27	0.26 ± 0.01	4.80	83.33
15	0.04	0.05	0.05	0.05 ± 0.01	7.33	92.64	0.11	0.10	0.12	0.11 ± 0.01	9.22	92.94
20	0.02	0.01	0.02	0.02 ± 0.01	7.72	97.05	0.07	0.06	0.06	0.06 ± 0.01	8.93	96.15
25	BDL	BDL	BDL	BDL	-	-	0.03	0.03	0.03	0.03 ± 0.01	3.00	98.07
Harvest	-	-	-	ND	-	-	-	-	-	ND	-	-
Kinetic equation	Y= 0.0762x + 2.8424						Y= 0.0676x + 3.1213					
R^2 value	0.988						0.993					
Half-life	3.95 days						4.45 days					
PHI	-						1.79 days					

ND- Not Detected, BDL- Below Detectable Level (0.01 mg kg^{-1}), PHI- Pre-Harvest Interval, SD- Standard deviation

Table 7. Dietary risk assessment of imidacloprid in grape berries at 53 g a.i ha^{-1} and 106 g a.i ha^{-1}

53 g a.i ha^{-1}						106 g a.i ha^{-1}					
Days after treatment	Imidacloprid residues* (mg kg^{-1})	Dietary risk assessment				Days after treatment	Imidacloprid residues* (mg kg^{-1})	Dietary risk assessment			
		Male (65kg)		Female (55kg)				Male (65kg)		Female (55kg)	
		EDI (mg kg^{-1}/ bw/day)	Risk quotient (RQ)	EDI (mg kg^{-1}/ bw/day)	Risk quotient (RQ)			EDI (mg kg^{-1}/ bw/day)	Risk quotient (RQ)	EDI (mg kg^{-1}/ bw/day)	Risk quotient (RQ)
0 (2hrs)	0.68	0.001569	0.0262	0.00185	0.0309	0 (2hrs)	1.56	0.003600	0.0600	0.00425	0.0709
1	0.47	0.001085	0.0181	0.00128	0.0214	1	1.07	0.002469	0.0412	0.00292	0.0486
3	0.43	0.000992	0.0165	0.00117	0.0195	3	0.74	0.001708	0.0285	0.00202	0.0336
5	0.37	0.000854	0.0142	0.00101	0.0168	5	0.70	0.001615	0.0269	0.00191	0.0318
7	0.22	0.000508	0.0085	0.00060	0.0100	7	0.43	0.000992	0.0165	0.00117	0.0195
10	0.11	0.000254	0.0042	0.00030	0.0050	10	0.26	0.000600	0.0100	0.00071	0.0118
15	0.05	0.000115	0.0019	0.00014	0.0023	15	0.11	0.000254	0.0042	0.00030	0.0050
20	0.02	0.000046	0.0008	0.00005	0.0009	20	0.06	0.000138	0.0023	0.00016	0.0027
25	BDL	-	-	-	-	25	0.03	0.000069	0.0012	0.00008	0.0014
30	BDL	-	-	-	-	30	BDL	-	-	-	-

EDI-Estimated Daily Intake, BDL- Below Detectable Level, * Mean of three replications

4. Conclusion

The present study confirms that residues of imidacloprid in grapes were below LOQ after treatment irrespective of doses. We conclude that reduction of pesticide residue depends on not only the behaviour and physiochemical properties of the pesticides and also climatic conditions prevailed during experimental study, The toxicological dietary risk assessment data shows that risk quotient is less than one which confirms safer to consumers. Pre-harvest interval does not apply for imidacloprid at recommended dose being as grapes harvested on the day of application itself were found safe for consumption and PHI/ safe waiting period of 2 days is suggested for double dose for harvesting.

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Fig.1 Dissipation curve of imidacloprid 17.8 SL in grapes