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Field Bioefficacy and Residue Dynamics of Most Effective Insecticides Against Aphids in Taro (*Colocasia esculenta* (L) Scott, 1832)

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ABSTRACT

Tuber crops including aroids fulfil ample demands of global food supply and utilized as feed of animals along with processed products for human consumption. Aphid (*Aphis gossypii*), an oligophagous pest, known to cause considerable qualitative and quantitative losses to the cultivation of aroids in general and taro in particular. This study reports the field bio-efficacy of some insecticides of plant and chemical origin against aphid as well as residue dynamics of most effective insecticides in leaves and tubers of taro. For residue analysis QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) methodology was standardized for extraction and clean up followed by estimation through UHPLC (Ultra High-Performance Liquid Chromatography). Among different insecticides evaluated, single spray of imidacloprid both at single and double doses was found most effective in managing the aphid population with a remarkable higher tuber yield. The initial deposit of imidacloprid in the leaves of taro was 0.65 and 1.32 mg kg $^{-1}$ with half-life value of 1.04 and 1.41 days at single and double dose, respectively. The dietary exposure of the measured residues was found lower than the maximum permissible intake (MPI) of 0.33 mg person^{-1}day^{-1} on all the sampling days at both the doses. However, considering the default Maximum Residue Limit (MRL) of imidacloprid as 0.01 mg kg^{-1} on taro leaves and safety of the consumers the pre harvest intervals of 7 days may be suggested for effective dose following good agricultural practices.

Keywords: Neonicotinoid, sucking pest, management, phytotoxicity, dissipation, safety evaluation.

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INTRODUCTION

Taro, (*Colocasia esculenta* (L.) Scott) is a popular tuber crop commonly grown in India and other parts of South Asia, Africa, South Europe, South America and many oceanic island (Rao, 2010). Globally it ranked fourteen among the staple vegetable and occupies an area of about 10.8 million hectare in which about 1.5 million hectare is shared by Asia (FAO, 2010). It is primarily cultivated in eastern and north eastern states of India such as Bihar, Chhattisgarh, Odisha, West Bengal, Assam, & Uttar Pradesh. Bihar, owing to its geographical and ecological conditions, remains in folded condition during the month of May to July in almost every year. Because of this, there is always a deficit of vegetable production during this period of the year. At the same time, being tolerant to water logging conditions, taro act as a substitute for other vegetables. In, Bihar, it occupies an area of about 1367 hectare with production of 18864 metric tonnes annually and productivity of 13.80 metric tonnes per hectare (Annonymous, 2019). Both leaves and roots are edible and considered to be an important source of nutrients, rich in carbohydrate, proteins, and dietary minerals (Onwueme, 1978; Temesgen & Retta, 2015). It is also an important source of micronutrient like iron (Fe), cupper (Cu), magnesium (Mg), potassium (K), and zinc (Zn) (Gupta, Kumar, Tomer, Kumar, & Saini, 2019). Being a rich source of dietary fibre, it is used for the treatment of various diseases like high blood sugar, cancer, obesity, and problems related to alimentary canal (Saldanha, 1995). The leaves of taro are rich source of protein including minerals such as calcium, phosphorous, irons, and vitamins like vitamin C and B complex (www.ndsu.edu). The leaves of taro are used for preparation of curries as well as for making some crispy snacks.

Though, the crop is known to provide shelters to a number of insect visitors, aphid (*Aphis gossypii)* and Tobacco caterpillar (*Spodoptera litura*) causes considerable economic losses to it (Bhattacharyya & Mandai, 2006). Aphid causes damage to taro plant directly by sucking the cell sap from under surface of leaves and indirectly by enhancing the development of black sooty mould fungus. Though, aphid causes significant reduction in quality of leaves that are consumed by people, there was always a negligence of management of this pest or some time it is managed by application of synthetic organic insecticides. Presently, insecticide of organophosphate and synthetic pyrethroid groups were greatly used by farmers (Simaremare, 2020). Use of these harmful chemicals in a non-judicious way has led to the problem of insecticide resistance, residue, and pest resurgence. Hence, use of insecticides having systemic action offer huge scope in controlling the insect pest as these are considered to be relatively safe to non-target organisms because of high vertebrate selectivity ratio.

Imidacloprid and thiamethoxam are the selective insecticides, and belong to the neonicotinoid group, a systemic class of insecticide known to provide excellent control of aphid, whitefly, thrips, jassids, and other sucking pest (Elbert, Haas, Springer, Thielert, & Nauen, 2008; Jeschke, Nauen, Schindler, & Elbert, 2011) due to its competitive binding to the neonicotinic acetylcholine receptors at post synaptic membrane (Casida & Durkin, 2013). Till date, there is not a single insecticide recommended for management of insect pest in taro. As imidacloprid and thiamethoxam are very much effective in controlling sucking pest of different crops and are already recommended for use against aphid in other crops, the present study evaluated the efficacy of both the insecticides for management of aphid in taro. Since the leaves of taro are mainly used as vegetable and the foliar application may leads to the problem of residues and no scientific publication is available on taro, hence it is essential to study the residue dynamics at different intervals to suggest suitable waiting period for safe consumption.

MATERIALS AND METHODS

Experimental site and raising of a crop

The trial was undertaken at the field of All India Co-ordinated Research Project on Tuber Crops (AICRP), Dr. Rajendra Prasad Central Agricultural University-848125, Pusa, Samastipur, Bihar, India for the period of three consecutive seasons *i.e.* during 2018-19, 2019-20, and 2020-21. The experimental site was located at latitude of 25.98^0 N, longitude of 85.61^{0}E and an altitude of 52.12 m above MSL with medium slope. The soil type was typically calcareous having pH 8.3 with loamy to sandy loam in texture and good water holding capacity. The available N, P_2O_5 and K_2O in the experimental field were 195, 32 and 125 kgha^{-1}, respectively. The average maximum temperature was 30. 2^{0}C, 30.65^{0}C and 29.5 ^{0}C whereas average minimum temperature was 18.8^{0}C, 19.3^{0}C and 18.3^{0}C during the three years (crop period) of study, respectively. The average relative humidity was 74% during the crop period 2018-19, 74% during the crop period 2019-20 and 80% during the crop period 2020-21. The field was prepared and levelled by ploughing with disc harrow followed by rotavator and cultivator Arvi (Variety-Rajendra Arvi 1) was planted during the second week of February in plot of size 5m×4 m having planting geometry of 50-30 cm. Except pest management practices, all other agronomical practices were followed to raise the healthy crops.

Experimental design and treatments imposition

The experiment was laid out in Randomised Block Design (RBD) having seven treatments and three replications. The insecticidal treatments for management of aphid consists of two doses of Imidacloprid 17.8 SL @ 25 and 50 g a.iha^{-1}, two doses of Thiamethoxam 25 WG @ 37.5 and 75 g a.iha^{-1} along with two botanicals (Neem oil and Cassava Leaf Extract @ 0.5 %) and an untreated control. The spraying of chemicals was done during the afternoon hour of a sunny day with little or no wind by using knapsack sprayer. The volume of the water used for spraying was 500 Lha^{-1}. The infestation of aphid was noticed during the last week of May which was increased with time and crossed the Economic Threshold Level (ETL) during second week of June when the crop was of 112 days old. At the time of spraying, the crop was in vegetative stage and formation of tuber was also started.

Bio-efficacy against aphid in taro

The observation on aphid population was taken on one day prior to the treatments and 1, 3, 5, 7, and 10 days after the treatments. For that ten plants were selected randomly and population was counted from three leaves per each plant. At the end, the per cent reduction of pest over control was calculated as per following formula.

Per cent Reduction= [(control count-treatment count/control count) × 100]

Treatment wise corm and cormel yield were recorded after harvesting and were pooled, expressed in tonnes per ha.

Statistical Analysis

The statistical analyses were carried out by using *Statistical Package for the Social Sciences* (SPSS version 16.0). The data were pooled over the years and subjected to Analysis of Variance (ANOVA) as there were no significant interaction found among the treatments and years. The effects of the years were also non-significant. When ANOVA was significant, comparisons of significant means were made using least significant difference (LSD) at the probability level of 0.05.

Residue dynamic studies

Taro leaves samples (500 g) were collected from 9 different sites of each treated plot separately, at 0 (after 2 hours), 1, 3, 5, 7, and 10 days after treatment. As aphid is a pest of vegetative stages and the tubers were not formed properly at the time of treatments, tuber samples were collected 30 days after the treatment. Quick, Easy, Cheap, Effective, Rugged, Safe (QuEChERS) techniques with slight modification is used for processing of taro leaves and tuber samples for residue analysis (Anastassiades, Lehotay, Stajnbaher, & Schenck, 2003). The methodology was standardised by conducting linearity and recovery studies only. A macerated taro leaves and tuber sample (10 g) was transferred to 50 mL polypropylene centrifugal tube later kept it overnight in refrigeration. Samples were taken from refrigerator and 20 mL of acetonitrile (C_2H_3N) (HPLC grade) was added to each tubes. To each centrifuge tubes, NaCl (10 ± 0.1 g) was added and shaken for 10 min at 50 rpm on rotospin (Tarson®). Samples were centrifuged for 3 min at 2500 rpm. Moisture if any was removed from aliquot of acetonitrile by anhydrous sodium sulfate (Na_2SO_4) followed by cleanup through "dispersive solid phase extraction (DSPE)". For this, a polypropylene tube constituting "0.15 ± 0.01 g PSA sorbent, 0.90 ± 0.01 g anhydrous (MgSO4) and 0.05 ± 0.01 g graphitic carbon black" was prepared for an aliquot of 6 mL which was mixed thoroughly by vortex spinix (Tarson®). Again it was centrifuged for 3 min at 2500 rpm and finally a 3 mL aliquot was taken for residue analysis.

Ultra High-Performance Liquid Chromatography (UHPLC) was used for residue determination at wavelength 271 nm. The choice of mobile phase is very important for the separation of parent pesticide from co-extractives. Elution was performed in the gradient mode with the ratio 70:30 (HPLC grade acetonitrile: HPLC grade water) with the flow rate of 0.30 mL min^{-1}. 20ul samples were injected at ambient column temperature.

Risk evaluation through consumption of taro leaves was done by comparing the dietary exposure, *i.e.* theoretical maximum residue concentration (TMRC) vis-a-vis the maximum permissible intake (MPI). The values of the dietary exposure were calculated by multiplying the residue levels in each sample (mg kg^{-1}) with an average per capita consumption of taro leaves in Bihar which is about 0.023 kg $person^{-1}$ day^{-1}.

RESULTS

Bio-efficacy of imidacloprid against aphid in taro

The mean number of nymph and adult of aphid per plant before and after the treatment of insecticide were presented in Table 1**.** The aphid population prior to treatment varies non-significantly ($F_{cal\ (6,\ 36)} < F_{tab(6,\ 36)}$, Table 3.) from 24.93 ± 0.69 to 27.53 ± 1.16 in different experimental plots. After 10 days of treatment, significant reduction of aphid population was observed in plot treated with imidacloprid @ 50 g a.i.ha^{-1} (4.91 ± 0.19 aphid/plant) followed by plot treated with imidacloprid @ 25 g a.i.ha^{-1} (5.78 ± 0.68 aphid/plant). Thiamethoxam @ 75 and 37.5 g a. i. ha^{-1} was also found very effective in reducing the aphid population below Economic Threshold Level (ETL) i.e. 6.07 ± 0.47 and 6.67 ± 12 aphid/plant, respectively. Maximum reduction of aphid population was observed on 5^{th} days after treatment, thereafter there was slight rise in population in all the plots but was found below the ETL. Though all the chemical insecticides were found statistically at par in controlling the aphid population, imidacloprid @ 50 g a.i.ha^{-1} showed maximum reduction of aphid population. On the other hand, botanicals were found ineffective in suppressing the pest population below the ETL. However, in control plot, continuous rise in pest population was observed. Thus, one spray of imidacloprid at both the doses was considered sufficient to suppress the aphid population below ETL in taro.

Table 1. Effect of insecticidal treatments on aphid population in taro (pooled data of 3 years of experiment) .

Treatments	Dose (a.i/ha)	Aphids population/three leaves						PROC
		1 DBT	1 DAT	3 DAT	5 DAT	7 DAT	10 DAT	
Imidacloprid 17. 8 SL	25	*26.34 ± 1.57a	16.00 ± 0.18 e	10.33 ± 0.40c	3.98 ± 0.37 c	4.53 ± 0.23 de	5.78 ± 0.68 c	80.49
Imidacloprid 17.8 SL	50	27.04 ± 1.33 a	15.22 ± 0.34 e	9.18 ± 0.57c	2.78 ± 0.23 c	3.84 ± 0.04 e	4.91 ± 0.19 c	83.43
Thiamethoxam 25 WG	37.5	27.53 ± 1.16a	17.67 ± 0.54cd	11.36 ±0.52 c	5.40 ± 0.08 c	6.04 ± 0.24 c	6.67 ± 0.12 c	77.49
Thiamethoxam 25 WG	75	25.20 ± 1.68a	16.51 ± 0.29de	10.76 ±0.35c	4.67 ± 0.14c	5.71 ± 0.23 cd	6.07 ± 0.47 c	79.52
Cassava Leaf Extract	0.5%	26.31 ± 1.49a	20.04 ±0.17 b	15.31 ± 0.71b	12.69 ± 0.50 b	13.47 ± 0.37b	15.42 ± 0.49 b	47.97
Neem Oil	0.5%	24.93 ± 0.69a	18.89 ±0.72 bc	15.42 ± 1.34 b	11.73 ± 0.97 b	12.40 ± 0.74 bc	14.78 ± 0.87 b	50.13
Control	-	24.93 ± 0.79a	25.29 ± 0.83a	27.82 ± 0.71 a	28.67 ± 0.76 a	28.66 ± 1.25 a	29.64 ± 1.19 a	-
SE (m)±	-	1.17	0.49	0.80	0.90	0.51	0.69	-
CV(%)	-	7.77	4.62	9.71	15.54	8.20	10.10	-

Values followed by different letters in columns are significantly different at p = 0.05 by LSD

*Mean of three replication

DBT: Days before treatment

DAT: Days after treatment

PR: Per cent Reduction over control

Among the treatments, plot treated with imidacloprid @ 50 g a.i.ha^{-1} recorded maximum cormel yield of 15.58 ± 0.27 t ha^{-1} which was found statistically at par with plot treated with imidacloprid @ 25 g a.i.ha^{-1} (15.14 ± 0.10 t ha^{-1}). Similarly maximum total (corm + cormel) yield was recorded in the plot treated with imidacloprid @ 50 g a.i.ha^{-1} (26.41 ± 0.70 t ha^{-1}) followed by plot treated with imidacloprid @ 25 g a.i.ha^{-1} (25.41 ± 0.22 t ha^{-1}) and thiamethoxam @ 75 (25. 16 ± 0.35 t ha^{-1}) and 37.5 g a. i.ha^{-1} (24.85 ± 0.40 t ha^{-1}) (Table 2).

Table 2. Effect of insecticidal treatments on yield and yield attributes in taro (pooled data of 3 years of experiment).

Treatment	Dose (a.i/ha)	Cormel Yield (tha^{-1})	Corm Yield (tha^{-1})	Total Yield (tha^{-1})
Imidacloprid 17. 8 SL	25	15.14 ± 0.10 a	10.27 ± 0.31 ab	25.41 ± 0.22 ab
Imidacloprid 17.8 SL	50	15.58 ± 0.27a	10.83 ± 0.43 a	26.41 ± 0.70 a
Thiamethoxam 25 WG	37.5	14.89 ± 0.30 a	9.96 ± 0.33 b	24.85 ± 0.40 ab
Thiamethoxam 25 WG	75	14.98 ± 0.17 a	10.18 ± 0.24 ab	25.16 ± 0.35 ab
Cassava Leaf Extract	0.5%	12.52 ± 0.44 b	8.60 ± 0.13 c	21.13 ± 0.38 c
Neem Oil	0.5%	12.78 ± 0.31 b	8.60 ± 0.07 c	21.38 ± 0.34c
Control	-	10.50 ± 0.34 c	7.19 ± 0.23 d	18.19 ± 0.54d
Standard error of the mean (±)	-	0.31	0.27	0.43
CV (%)	-	3.89	4.92	3.22

Values followed by different letters in columns are significantly different at p = 0.05 by LSD

Table 3. ANOVA Table for polled analysis.

Source	DF	Ftab (P = 0.05)	Fcal (P = 0.05)						Cormel Yield	Corm Yield	Total Yield
			1 DBT	1 DAT	3 DAT	5 DAT	7 DAT	10 DAT			
Year	2	3.259	2.692	2.786	1.609	0.031	1.062	1.159	1.171	1.036	1.746
Replication	2	2.364	1.484	0.449	0.331	0.553	1.827	0.530	0.702	0.288	0.433
Treatment	6	2.364	2.288	144.020	191.927	299.786	814.342	476.560	110.311	53.422	139.625
Year ×Treatment	6	2.033	0.688	1.080	1.179	0.955	1.892	0.198	0.484	0.297	0.489
Pooled Error	12	-	-	-	-	-	-	-	-	-	
Total	36	-	-	-	-	-	-	-	-	-	

$F_{cal\,(6,\,36)} > F_{tab(6,\,36)}$) : Significant otherwise Non-Significant

DBT: Days before treatment

DAT: Days after treatment

DF: Degree of Freedom

Persistence dissipation and safety evaluation of imidacloprid in taro

The correlation coefficient (r^2) for the linearity curve prepared by injecting different concentration of imidacloprid standards i.e 0.01, 0.05, 0.1, 0.5 and 1.0 in solvent and matrix matched was more than 0.99. By considering the signal to noise ratio of 3, the Limit of Detection (LOD) was calculated as 0.004. The Limit of Quantification (LOQ) was calculated by dividing the nanogram of standard injected giving 10 per cent full scale deflection by the milligram of sample injected giving no interference are the retention time of standard eluted and found to be 0.01 mg kg^{-1}, respectively. The per cent recoveries at LOQ, 5 X LOQ and 10 X LOQ were more than 80 per cent with relative standard deviation (RSD) within 20 per cent and hence the results are presented as such without any correction factor.

The extractable residues of imidacloprid 17.8 SL in the leaves of taro are presented in Table 4. The represented chromatograms of standard, control taro leaves sample are presented in Fig. 1. The mean initial deposit of imidacloprid was 0.65 and 1.32 mg kg^{-1} at single and double dose, respectively. More than 45 per cent of the initial deposit was dissipated after 1 day of spraying at both the doses. The residue of imidacloprid dissipated to below the LOQ of 0.01 mg kg^{-1} after 5 days of application in single doses whereas that of in double doses dissipated after 7 days of application. Taro tuber samples collected 30 days after treatment did not showed the presence of imidacloprid residues above LOQ of 0.01 mg kg^{-1}.

Table 4. Residues (mg kg-1) of imidacloprid in taro leave samples collected at different intervals.

Days after treatment	Residues following application @ 25 g a.i. ha^{-1} (mean ± sd)	Per cent dissipation	Residues following application @ 50 g a.i. ha^{-1} (mean ± sd)	Per cent dissipation
0 (2 hours after treatment)	0.65 ± 0.13	-	1.32 ± 0.06	-
1	0.35 ± 0.07	46.15	0.72 ± 0.10	45.45
3	0.09 ± 0.03	86.15	0.20 ± 0.07	84.85
5	< 0.01	-	0.12 ± 0.03	90.91
7	< 0.01	-	< 0.01	-
10	< 0.01	-	< 0.01	-
Taro				
(30 days after treatment)	< 0.01	-	< 0.01	-

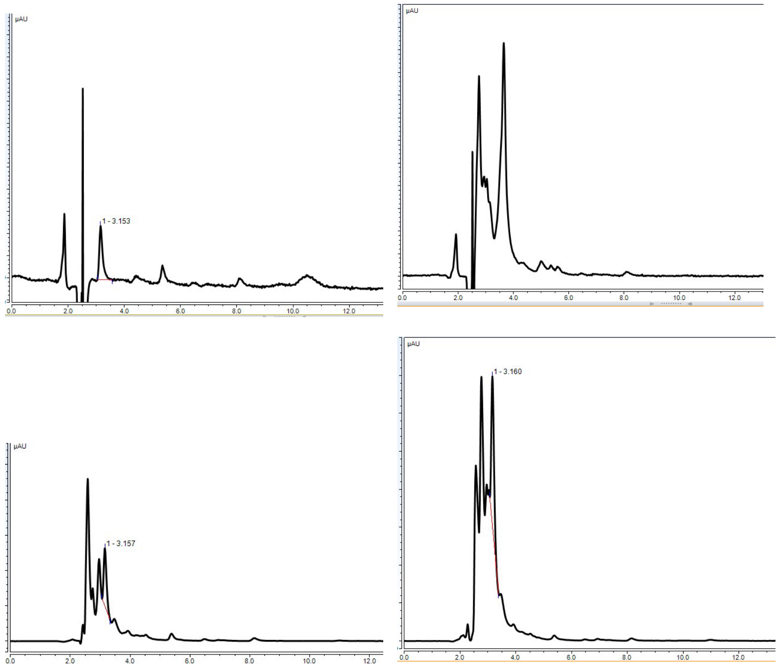

Fig. 1. Representative chromatograms a. standard imidacloprid @ 0.01 mg L^{-1} ; b. control taro leaves; c. single dose 0 day sample d. double dose 0 day sample

The degradation kinetics of the imidacloprid in leaves of taro was determined by plotting total residue concentration against time, and the maximum squares of correlation coefficients found was used to determine the equations of best fit curves. Confirmation of the first order kinetics was further made graphically from the linearity of the plots of log C against time (C= residues × 100). Half life ($t_{1/2}$) of imidacloprid was calculated (In 2/b value from the equation) as per formula given by (Hoskins, 1961). The dissipation parameters of imidacloprid in taro leaves are presented in Table 5. Fifty per cent of the initial deposits of imidacloprid dissipated within 1.05 (single dose) and 1.41 (double dose) days of field application. The day wise residue data had excellent fit to the 1st order models giving r^2 value of > 0.96. The Pre Harvest Intervals (PHIs) of imidacloprid were 6.33 and 9.71 days for single and double dose, respectively.

Table 5. Dissipation parameters of imidacloprid in taro leaves.

Parameters	Default MRL 0.01 (mg kg $^{-1}$)	
	Imidacloprid @ 25g a.i. ha^{-1}	Imidacloprid @ 50g a.i. ha^{-1}
r2	0.99	0.964
a (mg kg $^{-1}$)	1.819	2.069
B	-0.287	-0.213
DT50 (Days)	1.049	1.413
PHI (Days)	6.337	9.713
Y	1.819 – 0.287x	2.069 – 0.213x

b = Slope of regression line

a = Initial deposit obtained as in the regression equation

DT50 = Residual half-life (in days)

PHI (Pre Harvest Interval) = Time (in days) required for the pesticide residue to reach below the maximum residue limit of 0.01 mg kg-1

r2= Correlation Coefficient

In the state of Bihar, there is always short supply of vegetables during the month of June to August as on an average 7.18 per cent of total geographical area of the state was under flood or water logged situation affecting more than 7 lakhs people. During this time, taro act as a substitute for vegetable because of it's relatively tolerance to waterlogged conditions. Hence it is important to assess the safety associated with the use of imidacloprid. The risk evaluation of imidacloprid in taro leaves was evaluated as the MRL value is not available. For the risk evaluation, maximum permissible intake (MPI) was calculated as 0.33 mg person^{-1}day^{-1} by multiplying the Acceptable Daily Intake (ADI) (0.006 mg kg^{-1} body weight day^{-1}) value of imidacloprid with the average body weight of adult Indian (55 kg). The MPI value was compared with the TMRC. For calculation of TMRC, the maximum residues data of each day was multiplied with the daily consumption of taro leaves (0.023 kg) and found less that MPI on all the sampling days for single as well as the double dose (Table 6). The MRL for imidacloprid in taro leaves is not available, hence following the default MRL value of 0.01 mg kg^{-1}, PHI of 6.33 and 9.71 days for single and double dose, respectively may be suggested though TMRC were well below MPI for all sampling days. For taro tubers, PHI of 30 days may be suggested for safe consumption by following good agricultural practices.

Table 1. Effect of insecticidal treatments on aphid population in taro (pooled data of 3 years of experiment).

Interval (days)	Imidacloprid				
	MPIa for 55 kg person (mg person^{-1} d^{-1})	25 g a.i. ha^{-1}		50 g a.i. ha^{-1}	
		Maximum residues (mg kg^{-1})	TMRCb (mg person^{-1} d^{-1})	Maximum residues (mg kg^{-1})	TMRC(mg person^{-1} d^{-1})
0	0.33	0.79	0.0182	1.40	0.0322
1	0.33	0.44	0.0101	0.82	0.0189
3	0.33	0.13	0.0030	0.29	0.0067
5	0.33	<LOQ	-	0.16	0.0037
7	0.33	<LOQc	-	<LOQ	-

aADI = Acceptable Daily Intake

bTMRC = Theoretical maximum residue contribution

cLOQ= Limit of quantification (0.01 mg kg-1)

DISCUSSION

Since no published report on management of aphids in taro is available, the findings were not compared. However, the finding of (Khedakar, Bharpda, Patel, & Patel, 2012) was inconformity with present finding, who found that imidacloprid and thiamethoxam were most effective in controlling the mustard aphid whereas excellent control of wheat aphid by imidaclporid was also observed by (Joshi & Sharma, 2009) under field conditions. Since, no published work on residues of imidacloprid on taro leaves and tubers was found, hence the data observed were not compared with other published scientific reports. However (Yu et al, 2007) reported that no significant residues of imidacloprid was detected in leaves and stem of rice as well as in the expanded new leaf after 7 days of treatment agreeing our observation.

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