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Safety Evaluation and Persistence of Imidacloprid on Acid Lime (*Citrus aurantiifolia* Swingle)

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Acid lime is grown in almost all the states of India and occupies nearly an area of 1.06 lac ha. Lemon butterfly, leaf miner, psylla and black citrus aphid are the major insects which are responsible for citrus decline in India (Srivastava and Thakur1991; Gurung et al. 1993; Agarwala and Bhattacharya 1995; Bhattacharya and Dutta 1998; Singh and Singh 1998; Bhagat et al. 1999). Dimethoate, phosphamidon, diazinon, monocrotophos, quinalphos and methyl demeton etc. have been used for their control (Awasthi and Ahuja 1989; Anonymous 1997). These insecticides required higher amount per unit area and therefore likely to be retained in food and environment for a longer period thus affecting food chain and biodiversity. The presence of such concentrations of insecticides in edible foods assumes toxicological significance and consumption of such food might risk the health.

Imidacloprid, l-[(6-chloro-3-pyridinyl)-methyl]-4,5-dihydro-N-nitro-1H-imidazol-2-amine, a commercial example of the chloronicotinyl insecticides acting at the nicotinyl acetylcholine receptor (Bai et al. 1991; Moriya et al. 1992; Leicht 1993), is reported as highly active insecticide for homopteran pests (Iwaya and Tsuboi 1992; Shiokawa et al. 1994; Gourmet et al. 1996; JianZhong et al. 1996; Sannino 1997; Ramaprasad et al. 1998; Kumar et al. 2000a) and for some species of the order coleoptera, diptera and lepidoptera (Elbert et al. 1990, 1991). It has recently been registered in India for plant protection practices. Its bioefficacy and persistence has been studied on few crops like wheat, barley, rice, cotton, chilli, okra, mustard and sugar beet (Dewar and Read 1990; Rike et al. 1993; Ishii et al. 1994; Jarande and Dethe 1994; Rouchaud et al. 1994; Iwaya et al.1998; Kumar 1999; Kumar et al. 2000a; Dikshit et al. 2000). So far, studies on its residence time and persistence on acid lime following foliar spray treatment have not been conducted in Indian conditions. Therefore, this necessitated field experiments to examine (i) concentration of imidacloprid available in/on acid lime fruits at varied times after spray (ii) bioeffectivity against black citrus aphid, *Toxoptera aurantii* and (iii) risk assessment to humans.

MATERIALS AND METHODS

A three-season (3 year, 1998-2000) trial *viz*. summer (I), winter (II) and summer

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(III) was conducted. Imidacloprid (diluted from Confidor 200 SL, Bayer India Ltd., Delhi, India) was sprayed at the rate of 0.01, 0.025 and 0.05 per cent (0.5, 1.25, 2.5 mL L^{-1} , Confidor 200SL) on citrus trees at fruit setting stage (approximate size 1 cm dia.). One tree was sprayed with water to serve as control.

In order to determine the concentration of imidacloprid in/on fruits at various intervals, fruit samples were collected randomly from each treatment on 0 day (1 hr after insecticide spray) and 3, 5, 7 and 10 days after spray application, thereafter at five days interval upto 50 days. The fruit samples were cut into small pieces with a sharp knife to avoid squeezing, thoroughly mixed and then a sub-sample of 25 g was taken. Soil samples (0-10 and 10-20 cm deep cores were collected after fruiting season to examine the movement/leaching in the soil. Fruit and soil samples were extracted and cleaned as per the procedure of Kumar (1999) and Dikshit et al. (2000).

Concentration of imidacloprid in the cleaned extracts was determined by reverse product model Spectra system-2000 equipped with variable wavelength UV-150-UV-VIS detector and a Rheodyne injector (20 μl loop) connected to a Datajet reporting integrator. The stationary phase consisted of a Lichrosorb C-18 column (250 mm x 4.6 mm id) and the mobile phase was acetonitrile-water (40:60, v/v) maintained at a flow rate of 1 mL min^{-1} with detector wavelength at 270 nm.

Efficiency of extraction, clean up and determinative procedures was optimized through recovery experiments by fortifying untreated fruits and soil with insecticide. The efficiency of imidacloprid recovery from citrus fruit and soil was 89 to 91 and 82 to 83 per cent, respectively. The limit of detection was found to be 0.02 mg kg^{-1} for citrus fruits and 0.04 mg kg^{-1} for soil.

Bioeffectivity of imidacloprid on black citrus aphid population was recorded only from one season (season I, summer) on 30 randomly selected tender leaves. Observations were recorded from recommended treatment (0.025%) only. The aphid population was counted on 1, 3, 7 and 15 days after the foliar treatment.

In the absence of availability of Maximum Residue Limit (MRL) of imidacloprid on citrus fruits in India, an effort was made to assess the risk factor through consumption of contaminated fruits by establishing TMRC (Theoretical Maximum Residue Contribution) and MPI (Maximum Permissible Intake) values.

RESULTS AND DISCUSSION

The data on the amount of imidacloprid residues detected in/on acid lime fruits at varied time are presented in Table 1. The initial concentration of imidacloprid on acid lime fruits was 2.40 – 2.68 mg kg^{-1} and 3.10 – 3.60 mg kg^{-1} from 0.01 and 0.025 per cent treatments from all the three seasons. However, the initial concentration was 4.62 – 5.10 mg kg^{-1} in citrus fruits from highest rate of application. The observed high initial deposits may be due to high lipophilicity and systemicity of imidacloprid. The initial concentration on fruits declined from

Table 1. Amount of imidacloprid on acid lime from foliar spray treatments

Days	Treatment conc. (%)	Amount of imidacloprid (mg kg^{-1}) from 3 seasons		
		I	II	III
0 (1 hr)	0.01	2.40	2.68	2.58
	0.025	3.28	3.10	3.60
	0.05	4.62	4.82	5.10
3	0.01	1.48(38.33)	1.56(41.79)	1.41(45.34)
	0.025	2.15(34.45)	2.07(33.22)	2.36(34.44)
	0.05	2.75(40.47)	2.48(48.54)	3.00(41.17)
5	0.01	0.95(60.41)	1.10(58.95)	0.98(62.01)
	0.025	1.10(66.46)	1.24(60.00)	1.75(51.38)
	0.05	1.82(60.60)	1.50(68.87)	2.00(60.78)
7	0.01	0.64(73.33)	0.65(75.74)	0.67(74.03)
	0.025	0.78(76.21)	0.76(75.48)	1.00(72.22)
	0.05	1.00(78.35)	1.10(77.17)	1.18(76.86)
10	0.01	0.36(85.00)	0.38(85.82)	0.43(83.33)
	0.025	0.58(82.31)	0.48(84.51)	0.64(82.22)
	0.05	0.78(83.11)	0.82(82.98)	0.90(82.35)
15	0.01	0.20(91.66)	0.24(91.04)	0.30(88.37)
	0.025	0.30(90.85)	0.37(88.06)	0.34(90.55)
	0.05	0.60(87.01)	0.58(87.96)	0.78(84.70)
20	0.01	0.14(94.16)	0.17(93.65)	0.18(93.02)
	0.025	0.24(92.68)	0.20(93.54)	0.28(92.22)
	0.05	0.46(90.04)	0.40(91.70)	0.52(89.80)
25	0.01	0.10(95.83)	0.12(95.52)	0.09(96.51)
	0.025	0.18(94.51)	0.13(95.80)	0.19(94.72)
	0.05	0.38(91.77)	0.35(92.73)	0.42(91.76)
30	0.01	0.07(97.08)	0.04(98.50)	0.05(98.06)
	0.025	0.10(96.95)	0.10(96.77)	0.12(96.66)
	0.05	0.28(93.93)	0.22(95.43)	0.28(94.50)
35	0.01	ND (100.00)	ND (100.00)	ND (100.00)
	0.025	0.05(98.74)	ND (100.00)	0.06(98.33)
	0.05	0.22(95.23)	0.18(96.26)	0.16(96.86)
40	0.01	ND (100.00)	ND (100.00)	ND (100.00)
	0.025	ND (100.00)	ND (100.00)	ND (100.00)
	0.05	0.07(98.48)	0.06(98.75)	0.05(99.01)
45	0.01	ND (100.00)	ND (100.00)	ND (100.00)
	0.025	ND (100.00)	ND (100.00)	ND (100.00)
	0.05	ND (100.00)	ND (100.00)	ND (100.00)

I, summer; II, winter; III, summer; ND, not detectable; Figures in parentheses are percent cumulative loss.

2.40 to 1.48 mg kg^{-1} in 3 days, 0.64 mg kg^{-1} in 7 days, 0.20 mg kg^{-1} in 15 days and became nondetectable in 35 days from first season and lowest treatment rate. Similar decline in the concentration of imidacloprid was noticed from lowest treatment from the other two seasons. The insecticide residues reached to nondetectable concentration after 35 days from the first two treatments in all the seasons while after 40 days from highest application rate.

The reported concentration of imidacloprid declined progressively with the time lapse from all the treatments and seasons. There was no significant difference in dissipation of imidacloprid amongst the rates of application and seasons, suggesting that dissipation was independent of dose of insecticide and followed first order kinetics for dissipation. The data revealed that there is a rapid loss of imidacloprid from the fruits in the first few days after application i.e. upto 15 days and thereafter gentle decline in dissipation. It showed a twophase profile of dissipation behaviour of imidacloprid in/on fruits. The observed concentration dissipated more quickly during initial days after application, presumably due to loose association of insecticide with fruit skin and thereby quick loss of insecticide by environmental elements. The dissipation rate was lower at later stages. A possible explanation to this phenomenon can be attributed to the fact that as time passes by, the association of imidacloprid molecules with skin of the fruits became stronger in the sorbed form with time resulting in less and less imidacloprid being subjected to loss by weathering. The initial rapid loss followed by slow dissipation has been reported for many insecticides (Malathi et al. 1999; Khan et al. 1999; Kumar 1999; Kumar et al. 2000b).

Maximum Residue Limit (MRL) has not been fixed for this insecticide on citrus fruits in India. The MRL values varying from 0.5 to 1 mg kg^{-1} have been fixed for citrus fruit by different countries. India being a temperate country and to allow further safety assurance, if lowest value of 0.5 mg kg^{-1} is adopted, it would be seen that acid lime fruit contained residues below MRL after 15 days of spray treatments (Table 1). Therefore a waiting period of 15 days is suggested for this insecticide on acid lime fruits and consumption of fruit appears to be without appreciable risk.

To evaluate the risk to consumers, as acid lime is used as pickle (whole fruit), salad dressing, its juice in pickle and squash and as preservative, the Theoretical Maximum Residue Contribution (TMRC) through the consumption of acid lime treated @ 0.01, 0.025 and 0.05 per cent can be compared with Maximum Permissible Intake (MPI). The prescribed Acceptable Daily Intake (ADI) value of imidacloprid is 0.057 mg kg^{-1} body weight (Tomlin 1994). Multiplying ADI with the weight of average Indian, 55 kg, the MPI is obtained. The TMRC is calculated by multiplying the maximum residue detected in/on acid lime with the amount of citrus fruit consumed $person^{-1}$ day^{-1}. Since acid lime is used scantly in Balanced Indian Diet and therefore, data on the consumption of acid lime $person^{-1}$ day^{-1} is not available. Assuming that maximum 5 g of acid lime is consumed (pickle or juice) $person^{-1}$ day^{-1}, calculated TMRC on zero day (0.014, 0.019 and 0.027 mg $person^{-1}$ day^{-1}) were lower than MPI (3.135 mg $person^{-1}$ day^{-1}) calculated from toxicological data. Therefore, spray application of imidacloprid at the experimented level could be taken as safe from crop protection and environmental contamination point of view.

Imidacloprid residues were not detected in the soil samples (0-10, 10-20 cm) collected after fruiting seasons.

Table 2. Effectivity of imidacloprid on black citrus aphid

Treatment	Number of aphids per 30 leaves			
	Days			
	1	3	7	15
Imidacloprid	55	1	6	63
(0.025%)	(53.78)	(99.21)	(95.20)	(55.20)
Control	119	128	125	153

Figures in parentheses are percent reduction in aphid population

The bioeffectivity data on the black citrus aphid, *T. aurantii* is presented in Table 2. The insecticide gave 53.78 % aphid control after 24 hr (1 day) of application, while after 3 days an excellent control (99.21%) was noticed and remained effective thereafter upto 15 days but after one week aphid population started building up. The aphid population increased in the control and reached above Economic Threshold Level (ETL) after 15 days. Therefore, it appears that a second spray might be necessary at this stage if further population builds up.

To conclude, imidacloprid can safely be used as foliar spray treatments on acid lime for increasing yield and effective protection. TMRC values observed were lower than MPI, therefore, no appreciable risk appears to be involved and hence spray application of imidacloprid at the experimented levels could be taken safe from crop protection and environmental contamination point of view.

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