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Dissipation kinetics of imidacloprid residues in bhendi fruits

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Abstract
Imidacloprid was applied at 15, 25 and 50 g a.i. ha^{-1} in bhendi and the level of residue was assessed to arrive the safe pre harvest waiting period in bhendi fruits. The bhendi fruit samples (250 g) were collected at 0, 1, 3, 5, 7, 10 and 14 days after the last round of application. The imidacloprid residue in bhendi fruit sample was estimated by High Performance Liquid Chromatography (HPLC). The initial deposits of imidacloprid at 15, 25 and 50 g a.i. ha^{-1} were 1.722, 3.006, 3.677, respectively in the field experiment I and 1.500, 2.985, 3.778, respectively in the field experiment II. The residues gradually decreased and reached the below detectable level (BDL) on 7th day after treatment in both the experiments treated with imidacloprid 25 g a.i. ha^{-1} (X dose) and on 10th day in case of imidacloprid 50 g a.i. ha^{-1} (2X dose). The half life period of imidacloprid ranged from 1.33 to 1.55 days. A safe waiting period of three days may be recommended for harvesting bhendi fruits after application of imidacloprid at 25 g a.i. ha^{-1}.

Keywords: Imidacloprid, bhendi, dissipation, half-life, waiting period

Introduction
Bhendi, *Abelmoschus esculentus* (L.) Moench is one of the important vegetable crops grown throughout the tropical and warm temperate regions of the world. They are valued for its edible green fruit and the tender fruits are the rich sources of vitamins (A, B and C), iron, calcium, magnesium and also certain other minerals. They are suitable for cultivation as kitchen garden crop as well as on large scale purpose. In India, bhendi is cultivated in an area of 5.11 lakh ha with 58.49 lakh MT and 11.40 MT ha^{-1} production and productivity, respectively. In Tamil Nadu, bhendi is cultivated in an area of 13.11 thousand ha with production of 123.22 thousand MT and productivity of 9.40 MT ha^{-1} (Anonymous, 2017) [1].
Srinivasa Rao and Rajendra (2002) [16] recorded as high as 72 species of insects on okra, of which the pests *viz.*, okra shoot and fruit borer, *Earias* spp., aphids, *Aphis gossypii* (Glover), whitefly, *Bemisia tabaci* (Gennadius) and leafhopper, *Amrasca biguttula biguttula* (Ishida) causes significant damge to the crop. The sucking pests at early stage and fruit borers at late stage resulted in bhendi fruit yield reduction up to 69 per cent (Rawat and Sahu, 1973) [12]. The avoidable losses in yield due to okra fruit borer have been estimated as 36-90% (Misra *et al.*, 2002) [9].
Among the various strategies adopted by farmers to combat the pest menace, insecticides form the first line of defense in spite of their drawbacks. Several potent insecticides have been recommended for managing the sucking pests, but the arbitrary use of insecticides resulted in the development of resistance in insects to insecticides, resurgence, secondary pest outbreaks, disruption of natural enemy complex, loss in biodiversity and environmental pollution (Dhaliwal and Arora, 2001) [3]. To mitigate the yield losses due to insect pests in okra, huge quantity of pesticides were applied. The bhendi fruits are being harvested at shorter intervals are likely to retain unavoidably high level of pesticide residues which may be highly hazardous to the consumers. Chemicals may leave toxic residue in the harvested produce which is consumed by human beings (Babu Ramesh *et al.*, 1996) [2]. Some people are more vulnerable to impacts of pesticides than others. These problems have necessitated the search for environmentally and biologically safer pesticide molecules in bhendi. Chloronicotinyls/ neonicotinoids are the new group of crop protection agents highly effective against sucking pests which act on receptor protein of insect nervous system. The currently available insecticides acting on nicotinic acetylcholine receptors have no structural similarities with neonicotinoids. Hence neonicotinoids constitute the compounds with a new mode of action

(Leicht, 1996) [8]. Though chloronicotinyls are applied at a lower rate, even a small amount of insecticide residue is not acceptable. In bhendi, imidacloprid is most commonly used neonicotinoid compound by the farmers for the management of sucking pests. Hence, the present investigation has been undertaken to assess the dissipation kinetics and determine the waiting period of imidacloprid in bhendi.

Materials and Methods

Test insecticide

The technical material of the test insecticide imidacloprid, 1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine (Fig. 1) was used for quantification as well as to determine the detectable limits.

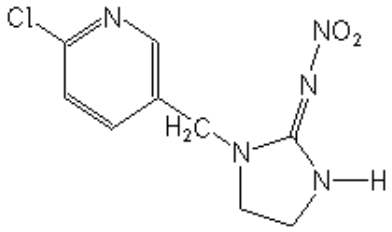


Fig 1: Structural formula of imidacloprid

The stock solution of 1000 ppm was prepared by dissolving 101 mg of imidacloprid technical material (99.0% purity) in 100 ml of acetonitrile (HPLC grade). From this stock, intermediate stock solutions of 100 and 10 ppm were prepared. Using 10 ppm stock, working standards of 0.5, 1, 2, 3, 5 and 10 ppm were prepared in HPLC grade acetonitrile.

Field experiments

Two field experiments were conducted at Maampalli and Madhampatti villages of Coimbatore District to determine the dissipation of imidacloprid residues in bhendi variety Ajeet 333. The insecticidal spraying was given twice using a hand operated Knapsack sprayer. The insecticide imidacloprid was applied at 15 g a.i ha^{-1} (½ dose), 25 g a.i ha^{-1} (X dose), 50 g a.i ha^{-1} (2X dose) and maintained along with an untreated check. Each treatment including control was replicated thrice.

Sampling

The samples (250 g bhendi fruits) were collected at 0, 1, 3, 5, 7, 10 & 14 days after treatment from each plot for determination of residues.

Extraction procedure

The bhendi fruit sample (20 g) was soaked in grade acetonitrile (50 ml) overnight, homogenized and filtered through Buchner funnel. After repeated washing, the pooled acetonitrile extract was evaporated to near dryness. The aqueous remainder was treated with 50 ml of saturated sodium chloride and 150 ml of hexane (three 50 ml portions) in a 500 ml separating funnel. After shaking well, the lower aqueous phase was collected and to which 100 ml of hexane: ethyl acetate (98:2 V/V) was added and shaken well. Once again, the lower aqueous phase was collected and partitioned with three 50 ml portions of dichloromethane. The pooled dichloromethane extract was passed through anhydrous sodium sulphate. The extract was evaporated to near dryness and the aqueous remainder was dissolved in ethyl acetate.

For column chromatography, 1.5 cm (id) x 50 cm (length) glass columns were used. Florisil® deactivated with 5 per cent water was used as an adsorbent at 4.5 g per sample. The drip tip of the chromatographic column was plugged with cotton wool. The Florisil® was slurried with 20 ml ethyl acetate and applied quantitatively into the column. This was sandwiched with two cm layers of anhydrous sodium sulphate. The column was prewashed with 20 ml ethyl acetate. The dry residue dissolved in small amount of ethyl acetate was poured on top of the column by means of a pipette and allowed to percolate. The active ingredient was eluted with 20 ml portions of acetonitrile (HPLC grade). The elutant was concentrated to near dryness, the residue dissolved in acetonitrile and fed into HPLC.

Quantification of residue

The final estimation of imidacloprid in bhendi fruits was done by High Performance Liquid Chromatography Hitachi model L 6200 using acetonitrile (HPLC grade): Water (HPLC grade) (35:65 V/V) as mobile phase and ODS 2 column. The flow rate was 1 ml min^{-1}, wavelength 270 nm and the quantity injected was 20 µl (fixed loop). The amount of residue was determined by comparing the sample response with the response of standard by using the formula.

$$\text{Residues in ppm} = \frac{H_s}{H_{std}} \times \frac{W_{std}}{W_s} \times \frac{V_{ex}}{V_s} \times \frac{A_s}{A_{std}}$$

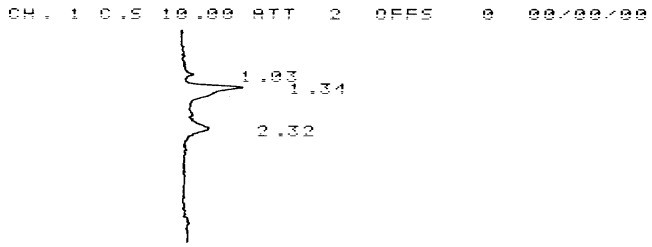
Where,

Table 1: Peak height of the sample

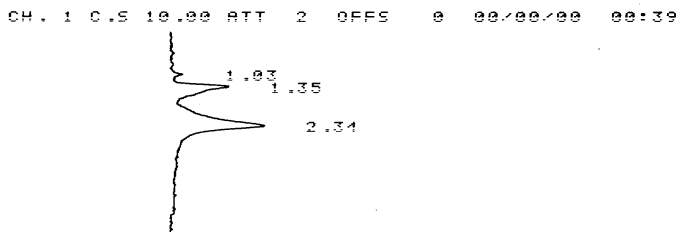
H_s	-	**Peak height of the sample**
H_{std}	-	Peak height of the standard
W_{std}	-	Weight of the standard injected in ng
W_s	-	Weight of the sample in g
V_{ex}	-	Volume of the final extract in ml
V_s	-	Quantity of the sample injected in µl
A_s	-	Attenuation of the sample
A_{std}	-	Attenuation of the standard

Results and Discussion

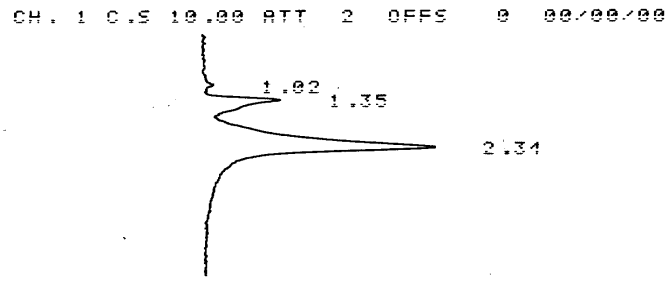
The recovery studies revealed that the mean recovery of imidacloprid from bhendi fruits was 93.72 per cent. The standard chromatogram is depicted in Fig 2. The initial deposit of imidacloprid was found to be 1.722, 3.006 and 3.677 µg g^{-1} from 15, 25 and 50 g a.i. ha^{-1} treatments, respectively in the first field experiment (Table 2). The residues progressively reduced with time and on 5th day the concentration was determined to be 0.060, 0.212 and 0.447 µg g^{-1} from respective treatments and became non detectable on 7th day from imidacloprid at 15 and 25 g a.i. ha^{-1} and 10th day from the double dose (50 g a.i. ha^{-1}). The half life values worked out for different doses *viz*., 15, 25 and 50 g a.i. ha^{-1} were 1.07, 1.33 and 1.34 days, respectively. Considering the maximum permissible residue limit (MRL) value of 0.7 µg g^{-1} for bhendi the suggested waiting period after spraying of imidacloprid at 15, 25 and 50 g a.i. ha^{-1} were 1.29, 2.68 and 3.40 days, respectively (Table 2).



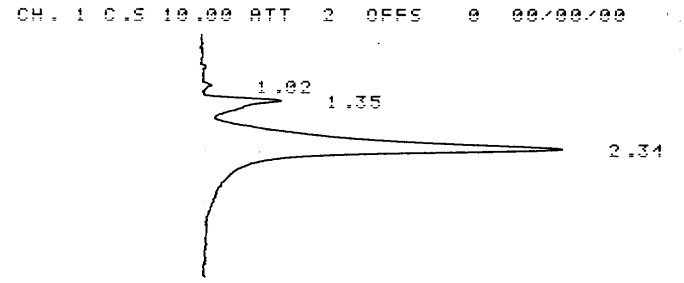
Imidacloprid 0.1 ppm



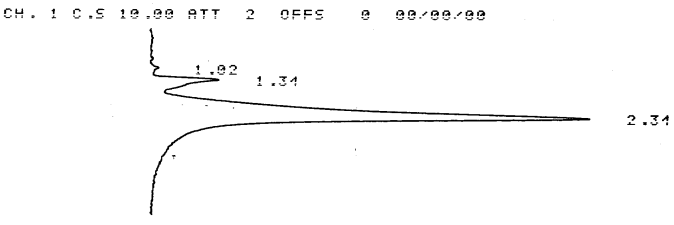
Imidacloprid 0.5 ppm



Imidacloprid 1.0 ppm



Imidacloprid 2.0 ppm



Imidacloprid 3.0 ppm

Fig 2: Standard chromatogram for imidacloprid in HPLC

In the second field experiment, following foliar application at 15, 25 and 50 g a.i. ha^{-1}, the initial deposits in/on the bhendi fruits were 1.500, 2.985 and 3.778 µg g^{-1}, respectively. The residues of different doses dissipated very fast and on 5th day 86.87 to 100 per cent dissipation was recorded. Residues were not detectable on 5th day from half dose (15 g a.i ha^{-1}) 7th day from recommended dose (25 g a.i ha^{-1}) and 10th day from double dose (50 g a.i ha^{-1}). The half life values were 1.63, 1.52 and 1.35 days and the suggested waiting period was 1.24, 2.92 and 3.38 days, respectively (Table 3) for imidacloprid 17.8 SL at 15, 25 and 50 g a.i ha^{-1}.

Table 2: Dissipation of imidacloprid in/ on bhendi fruits - Experiment I

DAT	Residues for 15 g a.i ha^{-1} (µg g^{-1})	Dissipation (Per cent)	Residues for 25 g a.i ha^{-1} (µg g^{-1})	Dissipation (Per cent)	Residues for 50 g a.i ha^{-1} (µg g^{-1})	Dissipation (Per cent)
0	1.722	-	3.006	-	3.677	-
1	0.971	43.61	2.107	29.91	2.905	21.00
3	0.452	73.75	0.987	67.17	1.682	54.26
5	0.060	96.52	0.212	92.95	0.447	87.84
7	BDL	100.00	BDL	100.00	0.096	97.39
10	BDL		BDL		BDL	100.00
Half life (Days)	1.07		1.33		1.34	
Waiting period (Days)	1.29		2.68		3.40	
r value	0.976		0.982		0.975	
Regression equation	Y = 0.679 - 0.645x		Y = 1.243 - 0.521x		Y = 1.604 - 0.518x	

DAT - Days after treatment
BDL - Below detectable limit
MRL 0.7 ppm

Table 3: Dissipation of imidacloprid in/ on bhendi fruits - Experiment II

DAT	Residues for 15 g a.i ha^{-1} (µg g^{-1})	Dissipation (Per cent)	Residues for 25 g a.i ha^{-1} (µg g^{-1})	Dissipation (Per cent)	Residues for 50 g a.i ha^{-1} (µg g^{-1})	Dissipation (Per cent)
0	1.500	-	2.985	-	3.778	-
1	0.903	39.80	2.035	31.83	2.713	28.79
3	0.412	72.50	1.051	64.79	1.577	58.26
5	BDL	100.00	0.287	90.39	0.496	86.87
7	BDL		BDL	100.00	0.092	97.56
10	BDL		BDL		BDL	100.00
Half life (Days)	1.63		1.52		1.35	
Waiting period (Days)	1.24		2.92		3.38	
r value	0.998		0.986		0.975	
Regression equation	Y = 0.373 - 0.425x		Y = 1.180 - 0.457x		Y = 1.585 - 0.514x	

DAT - Days after treatment
BDL - Below detectable limit
MRL 0.7 ppm

Imidacloprid sprayed at 15, 25 and 50 g a.i. ha^{-1} left an initial deposits 1.722, 3.006 and 3.677 µg g^{-1} (Field experiment I) and 1.500, 2.985 and 3.778 µg g^{-1} (Field experiment II) in the bhendi fruits, respectively. Dissipation of initial deposits was 29.91 and 31.83 per cent on day one in the first and second field experiments, respectively for the recommended dose of imidacloprid (25 g a.i. ha^{-1}). The half-life of imidacloprid applied as foliar treatment at 25 g a.i. ha^{-1} was 1.33 to 1.52 days. The suggested waiting period calculated based on maximum permissible residue limit (MRL = 0.7 ppm) for bhendi was 2.68 to 2.92 days, respectively. The present results are in accordance with the findings of Sivaveerapandian (2000) [14] and Suganthy (2003) [17]. The residue of imidacloprid was detected only up to 3, 5, 3 and 3 days after treatment in bhendi, chilli, radish and mango, respectively (Suganthy, 2003) [17].

Dikshit and Pachauri (2000) [5] studied the persistence and bioefficacy of imidacloprid on tomato fruits and found that initial deposits of imidacloprid were found to be 1.35 and 2.40 mg kg^{-1} from 20 and 40 g a.i. ha^{-1} treatments, respectively. The imidacloprid residues progressively reduced with time and on the seventh day the concentration reported to be 0.08 and 0.18 mg kg^{-1} from respective treatments and became non detectable on tenth day from normal dose (20 g a.i. ha^{-1}) and the safe waiting period was seven days after treatment. Dikshit *et al.* (2000) [4] reported that the residues of imidacloprid in okra fruits were found to be 0.08, 0.10, 0.14 and 0.24 mg kg^{-1} from 3, 5.4, 10.8 and 21.6 g a.i. kg^{-1} seed treatments, respectively, after 55 days of sowing and became non detectable after 60 days of sowing.

According to Mukherjee and Gopal (2000) [10], the insecticide imidacloprid dissipated with a half-life of 3-5 days and persisted longer on mustard leaves, but the metabolites were found to be translocated up to 10 days in eggplant, cabbage leaves and mustard leaves. Kumar and Dikshit (2001) [7] indicated that imidacloprid (Gaucho® 70 WS, 5 and 10 g a.i. kg^{-1} seed and Confidor® 200 SL, 20 and 40 g a.i. ha^{-1}) was detectable up to 82 and 96 days in plants after sowing from lower and higher doses of seed treatment whereas it dissipated faster and became non detectable after 7 and 15 days of foliar treatments from lower and higher rates of application, respectively in mustard (*Brassica campestris* Linn.) and at harvest mustard grains did not contain imidacloprid residues. Kharbade *et al.* (2003) [6] evaluated the dissipation of imidacloprid residues in chilli fruits and found that in green chillies, initial residues were 0.38 and 0.56 ppm in spray treatment of 100 and 150 ml ha^{-1} respectively and these residues reached below detectable limit (BDL) of more than 0.05 ppm in 4.19 to 5.48 days.

The average initial deposits of imidacloprid on the sweet orange rind were found to be 1.12 and 2.33 mg kg^{-1}and in the case of pulp it was 0.13 and 0.32 mg kg^{-1}from treatment at the recommended and double the recommended dose, respectively (Singh *et al*., 2017) [13]. Studies on the dissipation of imidacloprid in okra fruits when applied at 20 g a.i. ha^{-1} revealed that the residue levels on 1, 3, 5 days after spray was observed as 0.688 mg kg^{-1}, 0.367 mg kg^{-1}, 0.197 mg kg^{-1}, respectively. The residues of imidacloprid went below detection limit within 7 days of application. The estimated half life ($t_{1/2}$) of imidacloprid in okra fruits was observed to be 2.094 days. The residue level went below MRL (0.7 mg kg^{-1} for okra) within 3 days at the recommended dose (Sneha Joshi *et al.*, 2019) [15]. This was in tune with the present findings. Pandit *et al.* (2016) [11] stated that the half life ($t_{1/2}$) ranged

between 2.66-3.28 days in okra leaf and 1.76-2.07 days in okra fruit imidacloprid is a safe insecticide to be used in vegetables like okra.

From the present investigation, it was concluded that in bhendi fruits dissipation of initial deposits of imidacloprid after one day was 29.91 and 31.83 in the first and second field experiments for the recommended dose of imidacloprid (25 g a.i. ha^{-1}). The half life of imidacloprid at 25 g a.i. ha^{-1} applied as foliar treatment was 1.33 and 1.52 days for field experiment I and II, respectively. The suggested waiting period calculated based on maximum permissible residue limit (MRL = 0.7 ppm) for bhendi was three days, respectively.

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