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Persistence and dissipation of neonicotinoid insecticides on chilli fruits

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RESEARCH ARTICLE

Abstract

A field trial was conducted to study the dissipation of neonicotinoid insecticides, imidacloprid and acetamiprid, on chilli fruits for the estimation of its half-life and waiting period. The samples of chilli fruits drawn at 0, 1, 3, 5, 7, 15, 21 days after spraying were quantified on gas liquid chromatography equipped with Ni ^{63} electron capture detector for acetamiprid and high performance liquid chromatography for imidacloprid. The residues of imidacloprid persisted up to seven days, whereas the acetamiprid persisted up to fifteen days of spraying. From the dissipation data, the half-life values of acetamiprid and imidacloprid were 2.27 and 2.08 days, respectively. A waiting period of 7.18 and 11.26 days were calculated for acetamiprid and imidacloprid in chilli.

Keywords: chilli, neonicotinoid, imidacloprid, acetamiprid, residue, dissipation

1. Introduction

Chilli (*Capsicum annuum* L.) is an indispensable spice used as basic ingredient in everyday cuisine all over the world. Broad mite (*Polyphagotarsonemus latus* Banks) and thrips (*Scirtothrips dorsalis* Hood) are the major biotic constraints in the chilli production, which together can cause a damage of 50% (Kandasamy *et al.*, 1990). Due to monoculture in chilli growing tracts, this sucking pest's build up is so high that the farmers have to resort to a minimum of 5 to 6 chemical sprays. Increased and non judicious use of these chemical insecticides resulted in resistant development and resurgence of pests followed by environmental pollution, contamination of groundwater, and the presence of residues in chilli fruits. Several studies have been reported on the contamination of pesticide residues in chillies. Kale *et al.* (2002) reported that out of 149 vegetable samples monitored, frequency of pesticide contamination was maximum in chilli (80%) due to the presence of dicofol and endosulfan. In another study conducted in Andaman and Nicobar islands, out of 42 chilli samples analysed 59.5% were detected with residues of various pesticides and 11.9% of the residue detected samples were having residues above maximum residue limit (MRL) (Swarnam and Velmurugan, 2013).

In a technical paper published by the Ministry of Primary Industries, New Zealand it was reported that out of 19 paprika samples analysed, 10 were contaminated with pesticides like ethion, carbendazim, cypermethrin, triazophos, imidacloprid and acetamiprid (MPI, 2012). The problem of insecticide residues in crop produces can be very well managed by observing proper waiting periods of insecticides on chilli crop. As the chilli fruits are harvested in 5-6 days interval, the pre-harvest intervals prescribed for each insecticide should be strictly observed to overcome the residue problem in harvested fruits. Chilli being consumed more as dried products rather than green chillies, the residue accumulation will be more on dry weight basis.

Acetamiprid and imidacloprid are newer class of insecticide molecules coming under neonicotinoid group and are increasingly used for systemic control of plant sucking insects replacing organophosphorus compounds and methyl carbamates. Neonicotinoid insecticides act as an agonist at the postsynaptic nicotinic acetyl choline receptors of insect nervous system. They are readily absorbed by plants and act quickly at low doses on piercing-sucking insect pests (aphids, leafhoppers and whiteflies) of major crops (Tomizawa and Cassida, 2003). The toxicity of neonicotinoid insecticides, especially imidacloprid, to bee population has been well documented by many authors. Sublethal doses have been shown to impair navigation, foraging behaviour, feeding behaviour and olfactory learning performance in (*Apis mellifera*). Imidacloprid also decreases the foraging activity and affects bee mobility and communication capacity (Bortolotti *et al.*, 2003; Medrzycki *et al.*, 2003; Yang *et al.*, 2008).

The efficacy of these insecticides against the sucking pests of chilli has been reported by several authors (Mishra *et al.,* 2005; Reddy *et al.*, 2005; Singh *et al.,* 2005; Varghese and Mathew, 2013). However, no information is available on the persistence of these neonicotinoid insecticides on chilli in the warm humid tropical climate. Hence a study has been conducted to assess the dissipation pattern of these insecticides on chilli fruits and to work out the safety interval in chilli fruit after application of these insecticides.

2. Materials and methods

Chemicals and reagents

Analytical standards of acetamiprid and imidacloprid were obtained from M/S Sigma Aldrich Pvt. Ltd. (Bangalore, India). All the solvents were of analytical reagent grade and were obtained from Merck India Ltd. (Mumbai, India) and redistilled before use. Florisil (60-100 mesh) for column chromatography (pesticide residue grade) was procured from Fluka (Bangalore, India). Anhydrous sodium sulphate used as drying agent was washed with acetone and activated at 650 °C at muffle furnace for 4 h before use. The commercial formulation of imidacloprid (Confidor 200 SL) and acetamiprid (Pride 20 SP) were obtained from Bayer Crop Science India Ltd. (Mumbai, India) and Dow Agroscience Pvt. Ltd. (Mumbai, India) respectively.

Experimental design

The field experiment was carried out at the instructional farm, College of Agriculture, Vellayani, India, adopting the standard agronomic practices of Kerala Agricultural University. A field trial was laid out in randomised block design replicated thrice with a plot size of 4 m^2 and a spacing of 45×45 cm. Foliar sprays of acetamiprid 20 SP at 20 g active ingredient (a.i.)/ha and imidacloprid 200 SL at 20 g a.i./ha were given twice at seven days interval during the fruiting stage using a hand sprayer. Control plots were maintained and sprayed only with water. Samples of green chilli fruits were collected from each plot at 0 (2 h after application), 1, 3, 5, 7, 10, 15, 21 and 30 days after the last spraying. 100 g sample of sprayed chilli fruits carefully harvested were brought to laboratory for further processing and residue estimation.

Method validation studies

The analytical method was validated in terms of limit of quantitation, linearity and recovery. Calibration curves are plotted to standardise the linearity in residue estimation. The lower concentrations (0.1 to 1 mg/kg) prepared from the working standard solutions of each of these two insecticides were injected in to the different analytical instruments viz. high performance liquid chromatograph (HPLC; Shimadzu Corporation, Kyoto, Japan) and gas liquid chromatograph-electron capture detector (GLC-ECD; Shimadzu Corporation) and a calibration curve of insecticides were prepared by plotting concentration vs. peak area. Recovery experiments were carried out to assess the efficiency of extraction and clean up procedures and to standardise the procedures for pesticide residue estimation. For conducting the experiment, control samples of chilli fruits were separately spiked at three different levels with the analytical standards of the insecticides. Percentage recovery and relative standard deviation (RSD) of the spiked pesticides were calculated for different extraction and clean-up methods adopted.

Extraction, clean-up and residue estimation

The laboratory procedures for pesticide residue (single residue method) analysis of chilli fruits sample involve the basic steps of extraction, clean up, identification, quantification and confirmation.

Imidacloprid

Chilli samples (100 g) were chopped and then macerated in stainless steel waring blender and a representative sample (10 g) was taken in a 250 ml conical flask. The sample was extracted sequentially thrice with 30 ml of acetonitrile by shaking for 30 min in a platform shaker (SK 300; Jeio Tech, Seoul, Korea). The contents were filtered through Buchner funnel (Rivera make, Mumbai, India) using Whatman no. 1 filter paper (Merck, Mumbai, India) into 500 ml jointed round bottom flask. The filtrates were combined and concentrated to about 10 ml using a rotary vacuum evaporator (Heidolph, Schwabach, Germany) and transferred to 500 ml separatory funnel (Rivera make). The extract was diluted with 100 ml distilled water to which 30 g NaCl has been added and then partitioned thrice using 25 ml hexane each time and the organic layers was discarded. The aqueous layer was again partitioned with 25 ml of hexane:ethyl acetate (98:2) mixture and the organic phase were discarded. The aqueous phase was extracted thrice using dichloromethane (50 ml each time) and the combined extract was collected after passing through anhydrous sodium sulphate. This was again concentrated to 5ml in a rotary vacuum flash evaporator.

The extract was cleaned up by column chromatography (Rivera make) using activated florisil PR (5 g) sandwiched between two layers of anhydrous sodium sulphate (2-3 g) in a glass column having 2.2 cm internal diameter and 60 cm length. The column was pre washed with 30 ml ethyl acetate and the concentrated extract was eluted with 30 ml of acetonitrile. The eluate was concentrated to near dryness in a rotary vacuum flash evaporator and the residues were dissolved in 1 ml acetonitrile (HPLC grade; Merck). The residues of imidacloprid were estimated in reverse phase HPLC (model Shimadzu LC 20AT; Shimadzu Corporation), with a C-18 column (Waters, Bangalore, India) and PDA detector (Shimadzu Corporation). The mobile phase was acetonitrile:water (35:65, v/v) with a flow rate of 1 ml/min. Detection was done at a wavelength of 270 nm with an injection volume of 20 µl (fixed loop). The retention time of imidacloprid was 5.96 min.

Acetamiprid

Chilli samples (100 g) were chopped and then macerated in stainless steel waring blender and a representative sample (10 g) was extracted with 100 ml acetone in a 250 ml conical flask. The extract was filtered through a glass funnel plugged with glass wool. Extraction was repeated two more times with 50 ml acetone each time. The combined filtrate was taken in 500 ml separatory funnel and concentrated to 5ml using rotary vacuum flash evaporator. The concentrated extract was taken in 500 ml separatory funnel and 100 ml distilled water containing 30 g sodium chloride solution was added and partitioned thrice with 50 ml hexane and the hexane layer was discarded. The extract was finally partitioned thrice with dichloromethane (100 + 50 + 50 ml). The combined dichloromethane layer was collected after passing through anhydrous sodium sulphate. The extract was concentrated on a rotary vacuum flash evaporator to about 5 ml and subjected to clean up for removal of co-extractives and impurities.

The extract was cleaned up by column chromatography using activated florisil PR (6 g) sandwiched between two layers of sodium sulphate (2-3 g) in a glass column having 2.2 cm internal diameter and 60 cm length. The column was pre-washed using 20 ml hexane and extract was eluted with 100 ml of hexane:acetone (1:1) mixture. The cleaned eluate was concentrated to near dryness in a rotary vacuum evaporator and the volume was made up to 5 ml with acetone and analysed on gas liquid chromatograph (model GC 2010; Shimadzu Corporation) equipped with Ni^{63} ECD fitted with capillary column (DB-1; Shimadzu Corporation) of 30 m × 0.25 mm i.d. × 0.25 µm film thickness. The sample was injected in a split mode with split ratio of 1:10. The injector and detector temperature was maintained at 250 and 300 °C, respectively. The column temperature was programmed at 160 to 270 °C at the rate of 5 °C per minute (8 min hold). Ultra-high purity nitrogen was used as carrier gas with a flow rate of 1.5 ml/min and linear gas velocity of 39.9 cm/sec. The retention time of acetamiprid was 16.2 min.

Statistical analysis

The half-life ($T_{½}$) as well as the time required to reach below tolerance level (T_{MRL}) were calculated using Hoskins formula (Hoskin, 1961):

$$T_{½} = \log 2 / K_1 \quad (1)$$

$$T_{MRL} = (\log K_2 - \log \text{tolerance}) / K_1 \quad (2)$$

Where K_1 is the slope of regression line, and $\log K_2$ is the apparent initial deposit obtained in regression equation.

3. Results and discussion

Calibration curves for imidacloprid and acetamiprid are given in Figure 1 and 2. Good linearity was found within the range of concentration for these two insecticides. The limits of quantitation for both the compounds were found to be 0.01 mg/kg.

When chilli fruits were fortified with 0.01, 0.05 and 0.1 mg/kg of imidacloprid, the mean recovery percentage were 93.30, 113.00 and 88.00, respectively, and the RSD were 9.17, 4.08 and 8.58%, respectively. The recovery of acetamiprid for the selected analytical procedure on chilli fruits was in the range of 94.7 to 95.89% at a concentration from 0.01 to 0.1 mg/kg (Table 1).

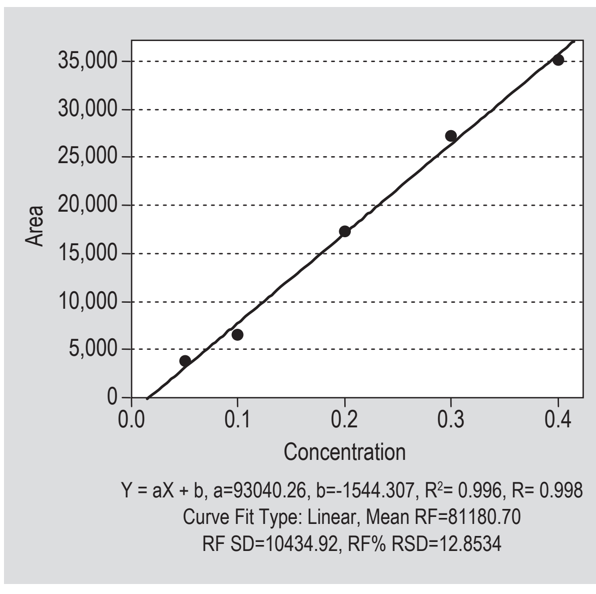

Figure 1. Calibration curve of imidacloprid (RF = response factor; RSD = relative standard deviation).

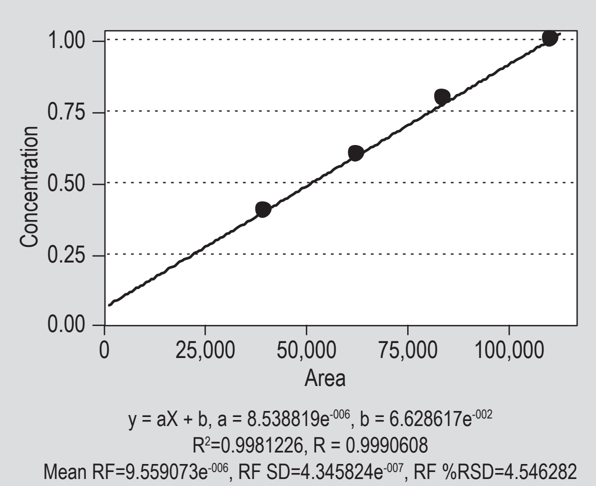

Figure. 2. Calibration curve of acetamiprid (RF = response factor; RSD = relative standard deviation).

Table 1. Fortification and recovery of imidacloprid and acetamiprid in chilli fruits.

No.	Fortification	Mean recovery % (relative standard deviation)	
		Imidacloprid	Acetamiprid
1	0.01	93.30 (9.17)	94.70 (16.73)
2	0.05	113.00 (4.05)	100.16 (20.17)
3	0.1	88.00 (8.58)	95.89 (22.44)

The data regarding initial residue deposits, dissipation percentage, regression equation, $T_{½}$ and waiting period of imidacloprid and acetamiprid are presented in Table 2 and log residues of imidacloprid and acetamiprid on chilli at different time intervals are presented in Figure 3.

Spraying of imidacloprid 20 g a.i./ha, left an initial average residue of 1.27 mg/kg on chilli fruits (Table 2). More than 60% of the residue got dissipated on the third day and about 90% of the residue got dissipated on the seventh day of spraying. The residues of imidacloprid were below detectable level on the tenth day of spraying. The dissipation data followed first order kinetics and the half-life calculated from the regression equation was 2.08 days.

Indumathi *et al.* (2001) reported that the residues of imidacloprid (Confidor 200 SL) at 0.3 and 0.6 ml/l dissipated exponentially with time in okra with a half-life of 2-4 days. In mustard leaves, imidacloprid applied at the rate of 20 g a.i./ha and 40 g a.i./ha had a half-life in the range of 3-5 days (Mukherjee and Gopal, 2000). In chilli a half-life of 2.66 days has been reported by Suganthy *et al.* (2010) which is in conformity with the present results. Based on the

Table 2. Residues of imidacloprid and acetamiprid in/on chilli fruits.[1]

Days after spraying	Mean residue ± standard deviation (dissipation %)	
	Imidacloprid	Acetamiprid
0 (2 h after spraying)	1.27±0.035	2.44±0.58
1	0.94±0.065 (25.99)	1.25±0.029 (48.78)
3	0.5±0.02 (60.63)	1.08±0.027 (55.74)
5	0.23±0.02 (81.89)	0.069±0.022 (71.73)
7	0.13±0.006 (89.77)	0.47±0.014 (80.74)
10	BDL	0.23±0.009 (90.58)
15	BDL	0.015±0.008 (99.39)
20	BDL	BDL
$T_{½}$	2.08	2.27
T_{MRL}	11.26	7.18
Regression equation	Y = 2.121 – 0.146x	Y = 2.432 – 0.133x
R	0.99	0.96
R^2	0.98	0.92

[1] BDL = below detectable level; R = correlation coefficient; R^2 = coefficient of determination; T½ = half-life; T_{MRL} = time required to reach below tolerance level.

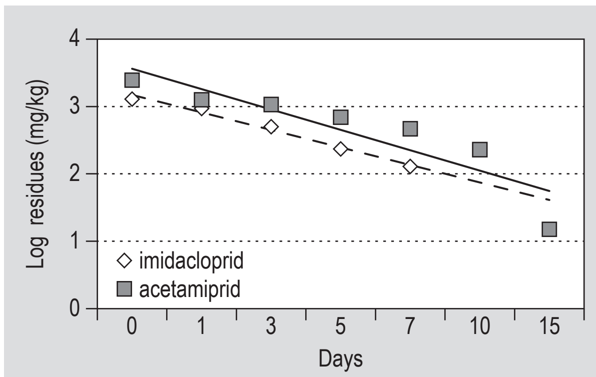

Figure. 3 Log residues of imidacloprid and acetamiprid at different intervals after spraying.

MRL value of 0.03 mg/kg fixed by the Prevention of Food Adulteration Act of 1954 in India, a waiting period of 11.26 days was calculated for imidacloprid on chilli. Hence it is recommended that green chillies can be harvested 12 days after application of imidacloprid for safe consumption.

The initial residues of acetamiprid on chilli fruits at the time of spraying was at the level of 2.44 mg/kg. The residues persisted up to fifteenth day in/on the fruits and on the fifteenth day 99.48% of the residues got dissipated. The residue of acetamiprid was below detectable level on 20^{th} day of spraying. In the present study the $T_{½}$ of acetamiprid on chilli a fruit calculated from the regression equation was 2.27 days. It was reported that the half-life value of acetamiprid was 1.02-1.59 days in the mustard plant (Pramanik *et al.*, 2006); 1.82 to 2.33 days in tea (Sharma *et al.*, 2008); and 1.9 to 2.5 days from zucchini and zucchini leaves (Park *et al.*, 2011).

The $T_{1/2}$ of acetamiprid 20 SP and acetamiprid 20 SL when applied at the rate of 20 g a.i./ha on chilli were 4.84 and 4.23 days, respectively (Sanyal *et al.*, 2008) and the variation from the present findings may be due to the difference in the agro-climatic conditions of locations in the studies. The persistence data showed that the degradation of acetamiprid was faster in the first week, with more than 70% of the residues got dissipated during this period. The remaining 30% of the residues got degraded in the second week of spraying. Based on the MRL value of 0.3 mg/kg fixed by the European Union, a waiting period of 8 days is recommended for the safe consumption of acetamiprid sprayed chilli fruits.

Chilli is the crop having short harvest intervals of 5-6 days where the selection of chemicals for plant protection plays a vital role in residue free produce. As per the present investigation, the waiting periods of the two chemicals selected for the study fits in to the harvest interval of chilli fruits and can be incorporated as a safe chemical component in the management of sucking pest complex of chilli.

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