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Residues and dissipation of fipronil and metabolites in Pomegranate fruits

■ D.R. KADAM*[1], B.V. DEORE[2] AND S.M. UMATE[3]

[1]Department of Agricultural Entomology, Vasantrao Naik Marathwada Krishi Vidhyapeeth, PARBHANI (M.S.) INDIA
[2]Department of Agricultural Botany, Vasantrao Naik Marathwada Krishi Vidhyapeeth, PARBHANI (M.S.) INDIA
[3]Department of Entomology, Mahatama Phule Krishi Vidyapeeth, Rahuri, AHMEDNAGAR (M.S.) INDIA

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***Corresponding author:**

ABSTRACT

Field and laboratory studies on residues and dissipation of fipronil and metabolites in pomegranate fruits were conducted during 2010 at the Pesticide Residue Analysis Laboratory, Department of Entomology, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar (M.S.) India. The studies revealed that residues of fipronil persisted up to 3 and 5 days in arils, 7 and 10 days in whole fruits and 10 and 14 days in peel of pomegranate fruits at recommended and higher doses, respectively. The metabolites showed interesting mode of dissipation.

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INTRODUCTION

Pomegranate (*Punica granatum*) is a native of Iran and one of the favorite table fruits of tropical and subtropical regions. India ranks first in area (0.12 million ha) and production (9.0 million tonnes) of pomegranate followed by Iran with an area of 0.065 million ha and production 8.00 million tonnes. In India, Maharashtra ranks first (0.096 million ha) contributing 70 per cent of the total area under pomegranate followed by Karnataka (0.013 million ha) and Andha Pradesh (0.0051 million ha). Again average productivity of pomegranate in Maharashtra is very less *i.e* only 6.2 t ha^{-1}. In Maharashtra, Nashik district has an area of 0.0354 million ha followed by Solapur 0.0310 million ha, Ahmednagar 0.00639 million ha and Sangli 0.00630 million ha. However, the productivity of this crop in India is only 7.4 t ha^{-1} which is significantly lower than other pomegranate growing countries like Spain (18.5 t ha^{-1}), USA (18.3 t ha^{-1}) and Iran (9.23 t ha^{-1}) (Anonymous, 2008).

The insect pests and diseases play significant role in reducing the productivity of this crop. The pomegranate crop (*Punica granatum* L.) suffers from the attack of several insect and non-insect pests. 86 species of insect pests infesting pomegranate have been reported from various parts of the world (Zirpe, 1966). Thrips, *Scirtothrips dorsalis* (H) contributes major losses in pomegranate cultivation, both qualitatively and quantitatively. In order to protect the crop from pest problems; farmers are spraying a number of chemical pesticides on this crop. The disadvantages of pesticides as known as 4R (Resistance, Resurgence, Risk and Residue) are well known. Since this fruit is mostly accepted as a table purpose fresh fruit, pesticide residues in this crop are of very much concern. Pesticide residues are also becoming a major obstacle in reducing India's export to foreign market. The export scenario shows that India contributes only 5 per cent of International market while Spain is most dominant with 80 per cent share. Keeping in view the above facts and figures the present study to study residues and dissipation of

spinosad in pomegranate fruits were carried out in the field experiment conducted in *Ambia* bahar (*Summer* season) of 2010. Pesticide residues were analyzed in peel, arils and whole fruits separately, collected periodically after the third spray to decide the safety of treatments to consumers.

MATERIAL AND METHODS

The field experiments on bioefficacy of newer insecticides against thrips, *Scirtothrips dorsalis* (H) of pomegranate were conducted during the *Ambia* bahar (*Summer*) and *Mrig* bahar (*Kharif*) seasons of 2010 on a five year old orchard of '*Bhagva*' variety at the Research Project on Arid Zone Fruits, Horticulture Farm, Department of Horticulture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar. Over all three sprays were given at an interval of 15 days, initiating first spray at the time of fruit setting. According to residue studies protocol prescribed by Central Insecticidal Board (CIB) two doses recommended (56.25 g a.i ha^{-i}) and double the recommended dose (112.5 g a.i ha^{-i}) were evaluated for analysis of residues. The marketable quality fruits of pomegranate weighing 1 kg were collected separately from each plot and packed properly in labeled polythene bags with rubber band and shifted to laboratory. Samples were collected at an interval of 0 (~ 2 hrs), 1, 3, 5, 7, 10, 14 and 21 days after last spray for residue analysis. From composite samples by quartering method after cutting, 50 g representative samples were taken for extraction, cleanup and estimation as described under each compound. The analytical procedure followed for fipronil and metabolites is as follows.

Extraction :

The residues were extracted in acetonitrile and vacuum filtered. The co-extractives were partitioned into 100ml hexane in 500ml separatory funnel by vigorous shaking for one minute and the hexane layer was discarded after phase separation. The solvent extract was drained into a 500ml round bottomed flask and concentrated to aqueous remainder on rotary vacuum evaporator at 40°C. The supernatant was decanted in a 50ml volumetric flask and the volume was adjusted to 50ml with water after adding 2ml isopropyl alcohol.

Cleanup :

Silica gel glass column was prewashed with 3ml methanol and 5ml of water. Sample extract (5 ml) was applied on to the column and discarded the eluate. The column was again rinsed with 5ml of water followed by 5ml of water: methanol (9:1). Fipronil residues were eluted with a 5ml collection volume of methanol. Methanol was removed on Turbo Vap LV concentrator and residues were dissolved in 3ml of toluene for estimation.

Estimation :

GC parameters :

Name of the instrument: Schimadzu Gas Chromatograph Model GC-2010 equiped with AOC-20 Auto injector and GC solution data software.

Gas chromatographic conditions :

Detector used	Electron Capture Detector (ECD)
Column used	DB-1, 30 m x 0.25 m x0.25 µm
Temperature parameters	250°C
Detector	300°C
Oven temperature	180^{o}c, 1 min hold, @ 1.5^{0}c/min 200^{0}c 18^{o}c/min 270°C
Carrier gas	Nitrogen
Gas flow rate	2.70 ml/min
Purge rate	3.0 ml/min
Make up	30 ml/min
Volume injected	1 µl
Retention time	Fipronil : 9.862 min MBO 6513 : 6.313 min. MBO 46163 : 13.691min MBO 45950 : 9.480 min

Calculations :

Calibration curve was prepared for spinosad by plotting the concentration of the calibration standards on X-axis and resulting peak height or area on Y-axis. Using regression analysis, the equation for the calibration curve was determined with respect to the X-axis. The concentration (C) of the analyte in the final solution was calculated from the measured peak height or area response (PR) and the least square co-efficient for the slope (m) and Y-axis intercept (b) as follows :

$$\mathbf{C = \frac{(PR - b)}{m}}$$

The concentration (ug g^{-1}) of the analyte in the sample was calculated from the concentration (C) in final volume (V), the weight (W) of the sample that was extracted, and the aliquot factor (AF) using the following equation :

$$\mathbf{\mu g\, g^{-1} \text{ or } ug\, g^{-1} = \frac{(C \times AF \times V)}{W}}$$

The aliquot factor was calculated from the appropriate extraction and aliquot volumes for each sample type,

$$\mathbf{AF = \frac{Total\ extraction\ volume}{Aliquot\ volume}}$$

The calibration of standards at various ng/ml levels showed good correlation between the concentration (X) and peak-height (Y) with the co-efficient of determination (r^2) averaging 0.955 or above.

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under the following heads :

Residues and dissipation of fipronil and metabolites in pomegranate fruits :

Arils :

The GC-ECD analysis of fipronil and metabolites involved extraction in acetonitrile and cleanup by silica gel column chromatography. At normal dose (25 g a.i. ha^{-1}) initial average residue of fipronil was 0.034 mg kg^{-1} on 0 DAS (Table 1). However, residues were not detected from 5th day onwards. The half-life (RL_{50}) value of 4.45 days indicated that the initial residue decreased to its half within 4.45 days. The time required for initial residues to reach below the detection limit was 3.12 days. The metabolites, MBO 6513, MBO 45950 and MBO 46136 were detected at 1 DAS only and the residue levels were 0.032, 0.022 and 0.028 mg kg^{-1}, respectively.

The immediate post application (zero time) initial deposit of fipronil in pomegranate arils was 0.064 mg kg^{-1} at 50 g a.i. ha^{-1}. Residues of fipronil were not detected in arils of pomegranate beyond 7 days. The residue half-life under field condition was 3.72 days. The time required for initial residue to reach below detection limit of 0.02 ppm was 6.38 days. The metabolites were detected only up to 5 DAS and were in

Table 1 : Residues of fipronil and metabolites in pomegranate fruits

Days after sprays	Fipronil and metabolites	Fipronil residues (mg kg^{-1})					
		25 g a.i. ha^{-1}	50 g a.i. ha^{-1}	25 g a.i. ha^{-1}	50 g a.i. ha^{-1}	25 g a.i. ha^{-1}	50g a.i. ha^{-1}
		Arils		Whole fruit		Peel	
0	Fipronil	0.034	0.064	0.064	0.104	0.110	0.197
	MBO6513	BDL	BDL	0.043	BDL	BDL	BDL
	MBO45950	BDL	BDL	BDL	BDL	BDL	BDL
	MBO46136	BDL	BDL	BDL	BDL	BDL	0.022
1	Fipronil	0.027	0.059	0.052	0.089	0.104	0.179
	MBO6513	0.032	BDL	0.056	BDL	BDL	BDL
	MBO45950	0.022	BDL	0.045	BDL	BDL	BDL
	MBO46136	0.028	BDL	0.054	BDL	BDL	BDL
3	Fipronil	0.021	0.034	0.042	0.068	0.082	0.140
	MBO6513	BDL	BDL	0.044	BDL	0.086	0.183
	MBO45950	BDL	BDL	0.039	BDL	0.075	0.025
	MBO46136	BDL	BDL	0.047	0.026	0.098	0.077
5	Fipronil	BDL	0.027	0.034	0.054	0.066	0.113
	MBO6513	BDL	0.031	BDL	0.059	0.073	0.147
	MBO45950	BDL	0.021	BDL	0.046	0.054	0.020
	MBO46136	BDL	0.027	BDL	0.058	0.073	0.051
7	Fipronil	BDL	BDL	0.022	0.033	0.049	0.089
	MBO6513	BDL	BDL	BDL	BDL	0.054	0.097
	MBO45950	BDL	BDL	BDL	BDL	0.041	0.076
	MBO46136	BDL	BDL	BDL	BDL	0.053	0.103
10	Fipronil	BDL	BDL	BDL	0.022	0.034	0.046
	MBO6513	BDL	BDL	BDL	BDL	BDL	0.023
	MBO45950	BDL	BDL	BDL	BDL	BDL	BDL
	MBO46136	BDL	BDL	BDL	BDL	BDL	0.033
14	Fipronil	BDL	BDL	BDL	BDL	BDL	0.021
	MBO6513	BDL	BDL	BDL	BDL	BDL	BDL
	MBO45950	BDL	BDL	BDL	BDL	BDL	BDL
	MBO46136	BDL	BDL	BDL	BDL	BDL	BDL
RL_{50} (Days)		4.45	3.72	4.88	4.41	5.77	4.75
T_{BDL} (Days)		3.22	6.38	8.11	10.65	14.58	15.20
T_{MRL} (Days)		12.12	13.82	17.88	19.46	26.11	23.93

LOQ : 0.02 µg g^{-1} BDL : Below detection limit, MRL : Maximum residue limit : 0.005 µg g^{-1}

the range of 0.021 to 0.031 mg kg^{-1}.

Whole fruit :

Fipronil and its metabolites residues were found in higher amounts in whole fruit as compared to arils (Table 1). The initial deposit of fipronil at normal dose (25 g a.i. ha^{-1}) was found to be 0.064 mg kg^{-1} at 0 DAS. Fipronil residues were found below detection limit at 10 DAS. The estimated half-life was of 4.83 days, whereas, residues reached below detection limit of 0.02 ppm in 7.99 days. The metabolite MBO 6513 was observed at a level of 0.043, 0.056 and 0.044 mg kg^{-1} at 0, 1 and 3 DAS, respectively. It was not detected in the samples collected at 5 DAS. The other metabolites MBO 45950 and MBO 46136 were found only in the samples collected on 1 and 3 DAS and the levels were 0.045 to 0.039 and 0.054 to 0.047 mg kg^{-1}, respectively.

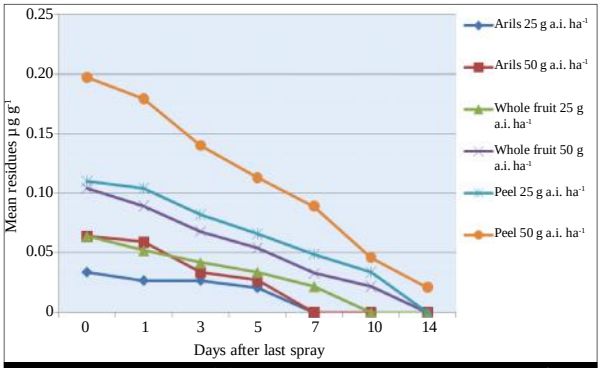
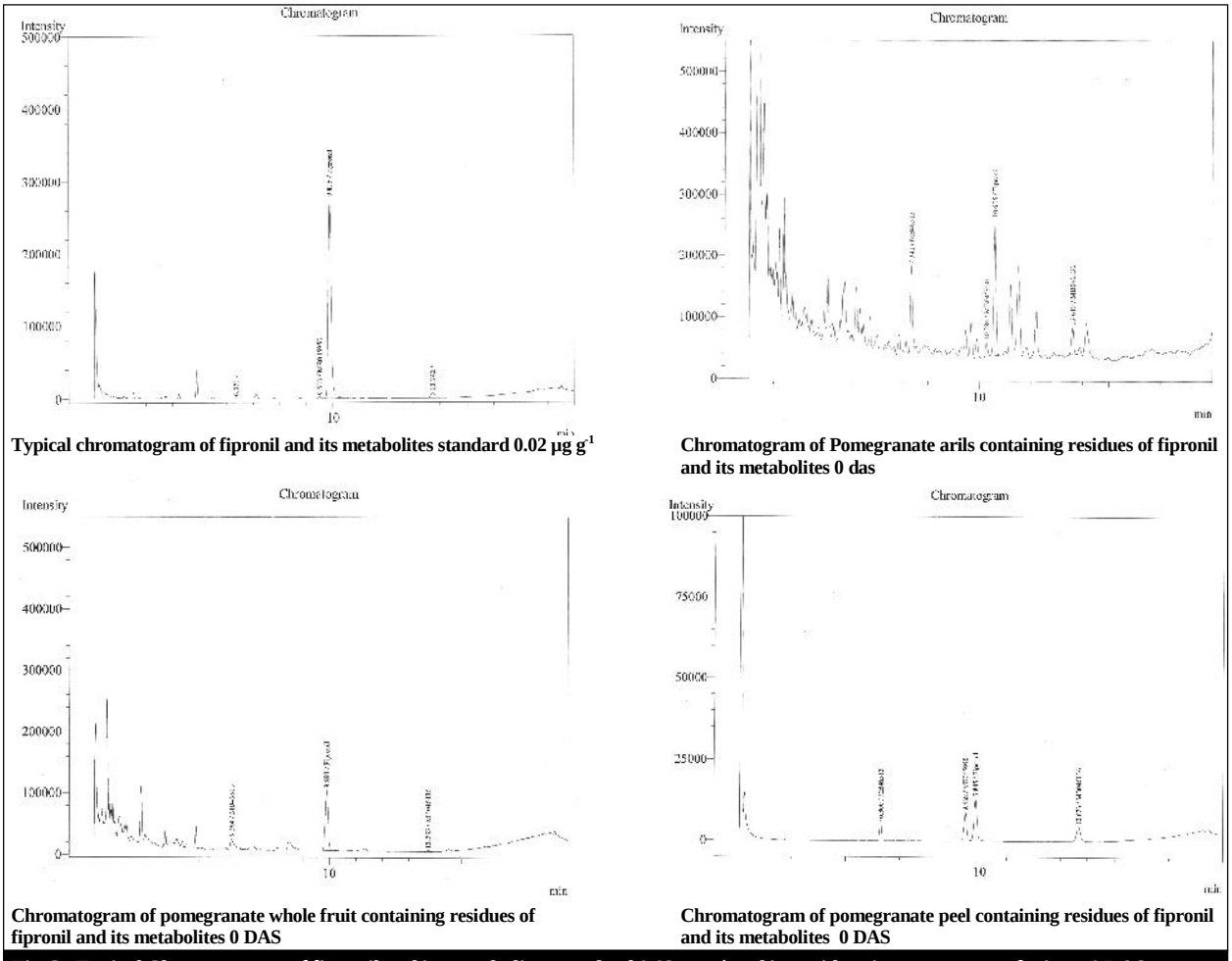


Fig. 1 : Residues of fipronil 5 SC (25 and 50 g a.i. ha^{-1}) in pomegranate fruits

At double dose (50 g a.i. ha^{-1}) the initial deposit of fipronil was 0.104 µg g^{-1} in whole fruit which dissipated to 0.089, 0.068, 0.054, 0.033 and 0.022 mg kg^{-1} at 1, 3, 5, 7 and 10 DAS, respectively. The metabolites detected in the samples collected at 5 DAS were in the range of 0.059 mg kg^{-1} (MBO 6513), 0.046 (MBO 45950) and 0.058 (MBO 46136).

Peel :

The samples collected from the plants treated with recommended dose of fipronil (25 g a.i. ha^{-1}) showed initial



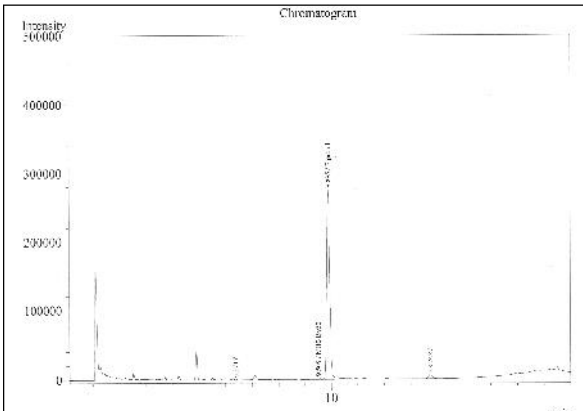
Typical chromatogram of fipronil and its metabolites standard 0.02 µg g^{-1}

Chromatogram of Pomegranate arils containing residues of fipronil and its metabolites 0 das

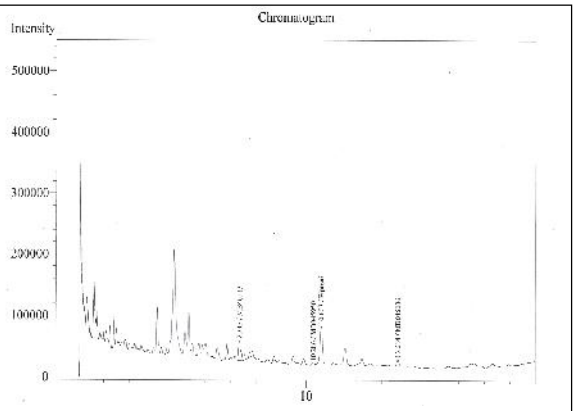
Chromatogram of pomegranate whole fruit containing residues of fipronil and its metabolites 0 DAS

Chromatogram of pomegranate peel containing residues of fipronil and its metabolites 0 DAS

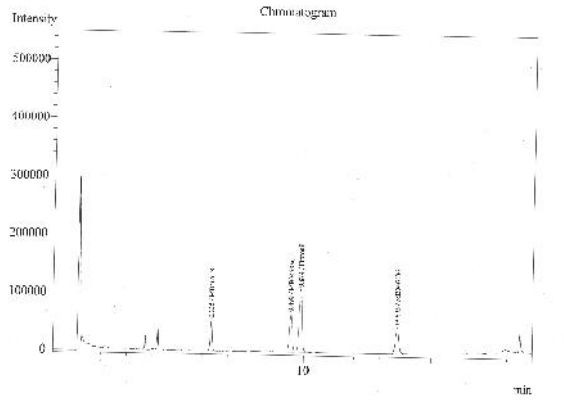
Fig. 2 : Typical Chromatogram of fipronil and its metabolites standard 0.02 µg g^{-1} and its residues in pomegranate fruits at 0 DAS



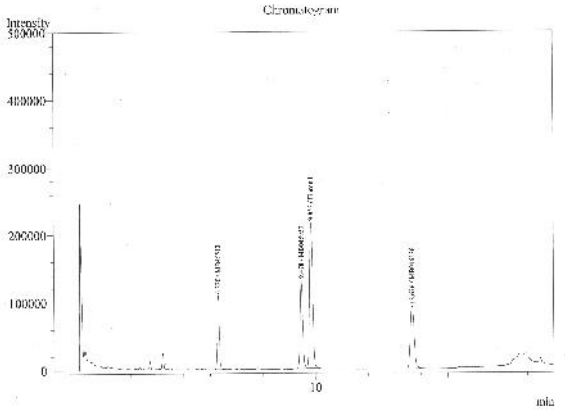
Typical chromatogram of fipronil and its metabolites 0.02 µg g-1



Chromatogram of pomegranate arils containing residues of fipronil and its metabolites 3 DAS



Chromatogram of pomegranate whole fruit containing residues of fipronil and its metabolites 3 DAS



Chromatogram of pomegranate peel containing residues of fipronil and its metabolites 3 DAS

Fig. 3 : Typical chromatogram of fipronil and its metabolites standard 0.02 µg g-1 and its residues in Pomegranate at 3 DAS

deposit of 0.110 mg kg^{-1}in peel (Table 1). The residues dissipated to the levels of 0.104 mg kg^{-1} (1 DAS), 0.082 mg kg^{-1} (3 DAS), 0.066 mg kg^{-1} (5 DAS), 0.049 mg kg^{-1} (7 DAS) and 0.034 mg kg^{-1} (10 DAS). The fipronil residues in peel of pomegranate took more than 10 days to reach below detection limit of 0.02 ppm with an estimated half-life of 5.72 days. The metabolite MBO 6513 was found in the range of 0.086 mg kg^{-1} (3 DAS), 0.073 mg kg^{-1} (5 DAS) and 0.054 mg kg^{-1} (7 DAS). The metabolite MBO 45950 was also found in the samples collected at 3, 5 and 7 DAS at the level of 0.075 mg kg^{-1}, 0.054 mg kg^{-1} and 0.041 mg kg^{-1}, respectively. The other metabolite MBO 46136 estimated from the samples collected at 3, 5 and 7 DAS showed residues of 0.098, 073 and 0.053 mg kg^{-1}, respectively.

The fipronil at double the recommended dose (50 g a.i. ha^{-1}) showed higher residues, initial residue at 0 DAS was observed to be 0.197mg kg^{-1}. The residues dissipated in pomegranate peel from 0.179 mg kg^{-1} to 0.021mg kg^{-1} within 14 days. The estimated half-life was 4.37 days and time required to reach the residues below detection limit was 15.20 days. The metabolites showed a variable dissipation pattern in pomegranate peel. The metabolite MBO 6513 was detected in the samples collected at 3, 5, 7 and 10 DAS at the levels of 0.183, 0.147, 0.097 and 0.054 mg kg^{-1}, respectively. The metabolite MBO 45950 was found in the samples collected at 3, 5 and 7 DAS at the levels of 0.025, 0.020 and 0.076 mg kg^{-1}, respectively. The metabolites MBO 6513 and MBO 45950 were not detected in the samples collected on 0 and 1 DAS. However, the third metabolite MBO 46136 was detected on (0.022 mg kg^{-1}) at 0 DAS. Interestingly, it was not detected in 1 day sample. Thereafter, the samples collected on subsequent days showed residue levels of 0.077 (3 DAS), 0.051 (5 DAS), 0.103 (7 DAS) and 0.033 (10 DAS) mg kg^{-1}, respectively.

In a study conducted by Stevens *et al.* (1998) in rice crop fipronil (12.5 g a.i. ha^{-1}) residue levels in the water column in small rice plots under glasshouse condition declined from 2.1 µg L^{-1} to 0.01 µg L^{-1} in 14 days after treatment. Kale (2003) reported initial deposit of fipronil on okra fruits at 15 and 30 g a.i. ha^{-1} to the extent of 0.17 and 0.3 mg kg^{-1}, respectively. Zhou *et al*. (2004) studied fipronil residues in vegetables under field condition in China and reported that

degradation of fipronil was faster in Pakchoi (half-life 2.6 days) than in soil (half-life 7.3 days). They also reported faster degradation of fipronil metabolites in soil. Dutta *et al*. (2008) standardized a method for the extraction and estimation of residues of fipronil and its three metabolites from cabbage and soil. They found that desulfonyl and sulfone derivatives of fipronil were the major metabolites found in the field samples of cabbage and soil which were formed after one day and reached their maximum amount on third day after application. The half-life of fipronil on cabbage varied from 7.5 to 7.6 days.

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