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Fate of Fipronil and its Metabolites in/on Grape Leaves, Berries and Soil Under Semi Arid Tropical Climatic Conditions

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Abstract Fate of fipronil and its major metabolites fipronil sulfide (MB 45950), fipronil-desulfinyl (MB 46513) and fipronil sulfone (MB 46136) were studied in/on grape leaves, berries and soil. As initial residue deposits on the leaves the major component was that of fipronil, while all the 3 metabolites were also present. Among metabolites residues of MB 46513 was highest followed by MB 46136 and MB 45950. In leaves fipronil degraded faster than its metabolites. The residues of fipronil in leaves degraded at the half-life of 9.6 and 18.3 days and that of total fipronil (sum of fipronil and its metabolites) at 13.6 and 20 days, from treatment at recommended and double the recommended dose, respectively. At the time of harvest in leaves, grape berries and soil residues of fipronil and all its metabolites were below the quantifiable limit of 0.01 mg kg^{-1}.

Keywords Fipronil · Metabolites · Half-life · Degradation

Fipronil {5-amino-1-[2,6-dichloro-4-(trifluoromethyl) phenyl]-4-[(trifluoromethyl)sulfinyl]-1H-pyrazole-3-carbonitrile} is a phenylpyrazole insecticide having broad spectrum activity against household and agricultural pests. It is one of the most persistent, lipophilic and toxic insecticides licensed for use since dieldrin, lindane and DDT. There are five degradation products of fipronil depending on the mode of dissipation and some of the metabolites are more toxic than the parent compound. The metabolite MB 46513 is about 10 times more acutely toxic to mammals than fipronil itself. The metabolite MB 461 is highly toxic to birds, and the metabolites MB 46136 and MB 45950 are more toxic to freshwater invertebrates than fipronil (Pesticide Action Network—UK (PAN) 2000). Fipronil is sold commercially under various trade names like regent, termidor etc. in India. It is found to be effective for control of various insect pests of vegetable crops like chilli, onion and okra especially the thrips (Jadav et al. 2004; Sinha et al. 2007; Reddy et al. 2007; Sule et al. 2003). Thrips (*Scirtothrips dorsalis*) is a severe pest of grapes, could cause 50%–100% marketable yield loss. It is found in major grape growing regions of India (Grape Profile, www.nrcgrapes.nic.in). Thrips are found throughout the year, but in epidemic form during flowering and early berry formation stage. Fipronil is used for control of grape thrips. Among all the fruit crops, grape (*Vitis vinifera*) has emerged as the most successful commercial crop in the recent years in India as also export of Indian grape to other Asian Countries, Europe and USA. This experiment has therefore been undertaken to study the fate of fipronil and its major metabolites on grape leaves, berries and soil in the semi arid tropical climatic conditions of India.

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Materials and Methods

Reference standards of fipronil (purity, 97.5%), its metabolites MB 46513 (purity, 97.8%), MB 45950 (purity, 98.8%) and MB 46136 (purity, 99.7%) were obtained from

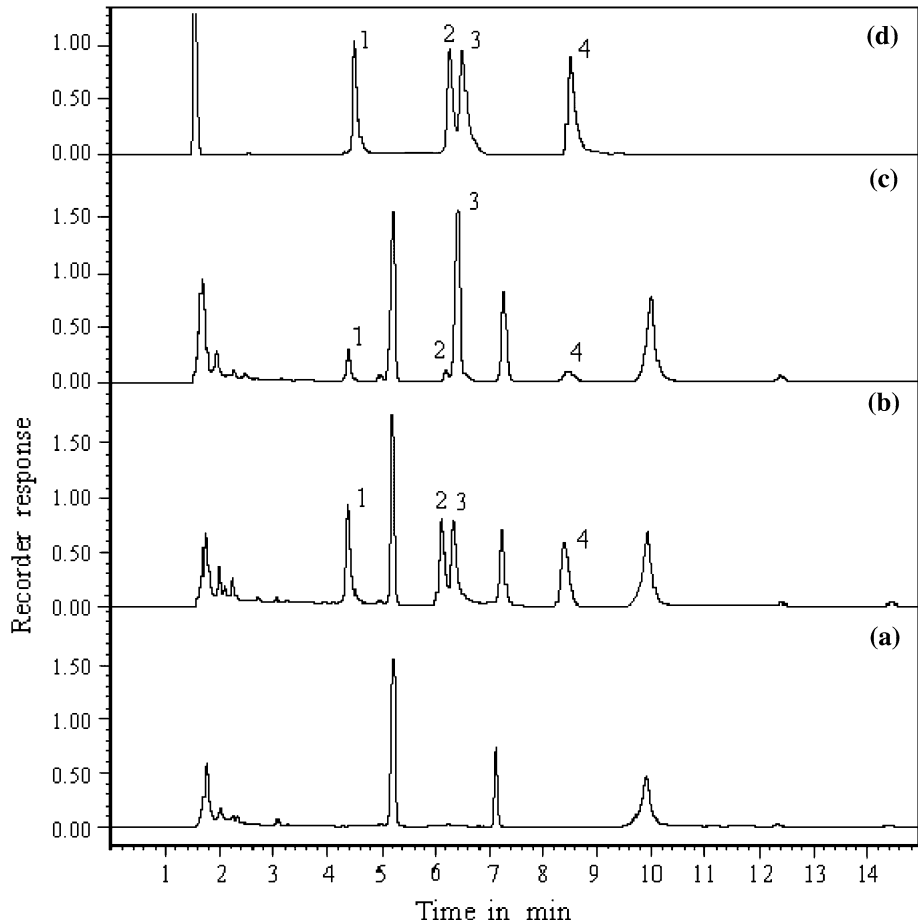

Fig. 1 GLC chromatogram of grape leaf, **a** untreated control; **b** fortified; **c** field sample after spray; **d** pure standard of fipronil and its metabolites (1. MB 46513; 2. MB 45950; 3. fipronil; 4. MB 46136)

Bayer Crop Science Ltd, India. All reagents and solvents used were of analytical grade.

Field experiment was conducted during October 2007–April 2008 at the experimental field of Indian Institute of Horticultural Research (IIHR), Bangalore, India, on 12 years old grapevines, variety Arka Neelamani. The treatments were untreated control, recommended dose of 62.5 g ha^{-1} and double the recommended dose of 125 g ha^{-1} of fipronil 80 WG. For every treatment 10 plants were selected. The spray volume taken was 1000 L ha^{-1}. Three treatment sprays were given at 15 day intervals starting from 50 days after pruning of the vines. The first treatment was given at berry setting stage and other two during fruit growth stage. Untreated control vines were sprayed with water. After the third spray analysis of leaf samples were carried out on 0 (2 h), 1, 3, 5, 7, 10, 15 day and at harvest (60 days after the last spray). Analysis of grape berries and field soil samples were carried out at the time of harvest.

From each of the 10 vines leaf samples were collected and pooled together. A total of 250 g leaf sample was collected, packed in plastic bags immediately transported to the laboratory. The leaves were mixed thoroughly and a representative 25 g sample in triplicates were processed for fipronil and its metabolites. From each vine a grape bunch weighing approximately 250 g was collected and pooled together. A total 2.5 kg grape sample was collected, mixed thoroughly and a representative 50 g sample in triplicates were processed for fipronil and its metabolites. From each plot soil samples were collected from 3 × 3 grid with total 10 sampling sites. From each treatment a total of 5 kg samples were collected, mixed thoroughly, air dried and passed through 2 mm sieve. A representative 100 g sample in triplicates were processed for fipronil and its metabolites.

Grape leaf and berries were homogenized and extracted with 100 mL 7:3 (v/v) acetonitrile-acetone and filtered under vacuum through a Buchner funnel. The container and the filter cakes were washed twice with 100 mL of the solvent mixture and the combined extracts were collected in a 500 mL flask. The acetonitrile-acetone fraction was concentrated under reduced pressure in a rotary vacuum evaporator. To the residues 30 mL saturated sodium chloride solution was added and the aqueous fraction was partitioned into 50 mL 1:1 (v/v) hexane–ethyl acetate 3 times. The hexane–ethyl acetate fraction was dried over anhydrous sodium sulphate, concentrated to 5 mL and passed through column with florosil as the adsorbent.

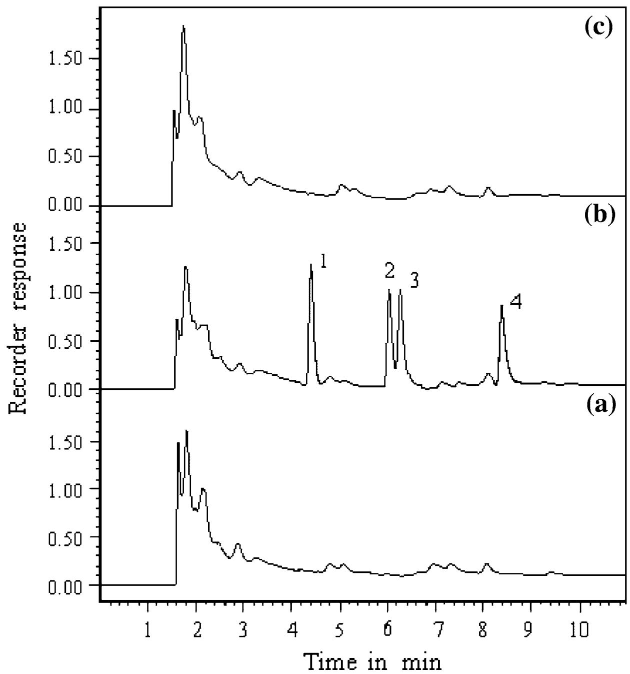

Fig. 2 GLC chromatogram of grape berries; **a** untreated control; **b** fortified; **c** field sample at harvest; (1–4 refer Fig. 1)

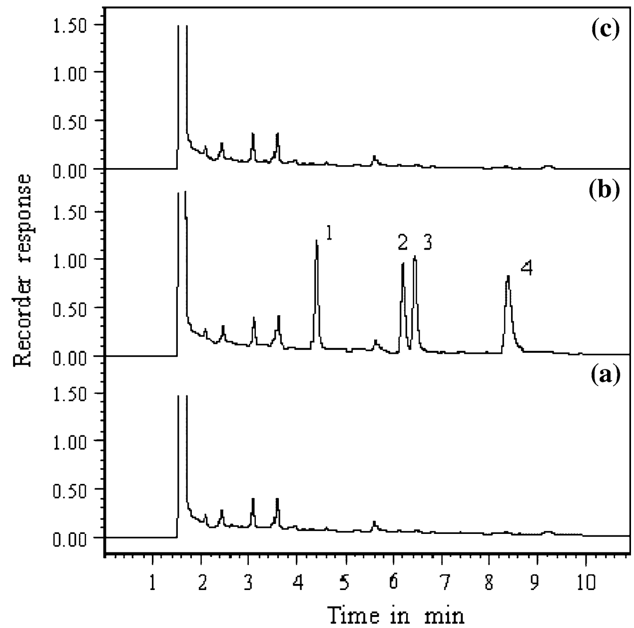

Fig. 3 GLC chromatogram of grape field soil; **a** untreated control; **b** fortified; **c** field sample at harvest; (1–4 refer Fig. 1)

Fipronil and all its metabolites were eluted with 100 mL of hexane–ethyl acetate, concentrated and redissolved in 5 mL acetone for analysis by GLC. Soil samples were processed in a similar manner. Fortification of fipronil and all its metabolites was carried out in leaf, grape and soil at the rate of 0.01, 0.1 and 1.0 mg kg^{-1} to validate the analytical method and get the recovery percent before the field samples were analysed.

A Shimadzu Gas Chromatograph GC-2010 equipped with electron capture detector (ECD) was used for residue analysis of fipronil and its metabolite in grape leaves, berries and soil. One micro litre of sample was injected with an auto sampler AOC-20i auto injector. A capillary column, Agilent DB-1 (30 m × 0.25 mm) was used and injector was kept at split mode with split ratio of 5. Ultra pure nitrogen was used as carrier gas at a flow rate of 1.0 mL min^{-1}. The column temperature was initially maintained at 220°C and programmed at 2°C min^{-1} to 240°C with hold time of 10 min. Injector and detector temperatures were maintained at 280 and 300°C. Under the above conditions the retention time of fipronil was 6.26 min; metabolite MB 46513—4.4 min; MB 45950—6.05 min; MB 46136—8.4 min. The chromatogram of pure standard, fortified, field sample of leaf, grape berries and soil are given in Figs. 1, 2, and 3.

Table 1 Recovery study of fipronil residues on leaf, grapes and soil at various fortification levels

Fortified concentration (mg kg^{-1})	Mean recovery (%)* ± SD			
	Fipronil	MB 45950	MB 46513	MB 46136
Leaf				
0.01	87.1 ± 0.66	88.34 ± 0.45	87.53 ± 1.24	88.78 ± 2.56
0.10	88.2 ± 3.23	88.23 ± 2.40	87.44 ± 2.68	88.54 ± 1.34
1.00	89.5 ± 2.17	89.11 ± 3.67	88.93 ± 3.82	88.54 ± 1.44
Grape				
0.01	84.4 ± 0.38	85.58 ± .20	85.66 ± 1.56	85.33 ± 2.32
0.10	84.5 ± 0.05	85.12 ± 2.47	86.22 ± 2.34	85.71 ± 2.64
1.00	85.5 ± 0.022	86.47 ± 3.53	85.98 ± 4.52	86.28 ± 2.54
Soil				
0.01	92.11 ± 0.02	91.88 ± 3.64	93.26 ± 1.24	92.33 ± 2.25
0.10	93.20 ± 0.23	92.40 ± 2.17	94.63 ± 2.52	93.45 ± 3.62
1.00	95.30 ± 0.12	93.02 ± 1.48	93.80 ± 1.26	95.78 ± 2.58

* Average of 3 replicates

Results and Discussion

The percent recovery of fipronil, its metabolites MB 46513, MB 45950 and MB 46136 in grape leaves, berries and soil are given in Table 1. In leaves recovery of fipronil and the metabolites were in the range of 87.1%–89.11%, in grape berries it was 84.4%–86.47% and soil 91.88%–95.78%. The limit of quantification of the method was 0.01 mg kg^{-1}.

In leaves as initial residue deposit the major component was the parent compound fipronil along with small amount of the other 3 metabolites (Table 2). Residues of fipronil, its metabolites MB 46513, MB 45950 and MB 46136 were 4.12, 0.27, 0.14 and 0.275 mg kg^{-1}, respectively at recommended dose. At double dose the residues were 6.56, 0.462, 0.28 and 0.472 mg kg^{-1}, respectively. From 0 (2 h) to the 3rd day fipronil seemed to get converted to its metabolites MB 46513 and MB 46136 as its concentration in the leaves reduced, but that of total fipronil (sum of fipronil and it's metabolites) did not reduce that much. The residues of fipronil degraded from 4.12 to 3.457 mg kg^{-1} by the 3rd day, whereas the residues of total fipronil reduced from 4.805 to 4.483 mg kg^{-1} only. Within this period the concentration of metabolites MB 46513 and MB 46136 in the leaves increased, MB 46513 being the major one. Its concentration increased from 0.27 to 0.493 mg kg^{-1} from 0 (2 h) to 3rd day. At double dose treatment concentration of fipronil had fallen from 6.56 to 5.22 mg kg^{-1} within the first 3 days, whereas the total fipronil had fallen from 7.774 to 7.29 mg kg^{-1} only. Concentration of major metabolite MB 46513 increased from 0.462 to 1.008 mg kg^{-1} during this period and MB 46136 from 0.472 to 0.857 mg kg^{-1}. Metabolite MB 45950 concentration was maximum on the initial day (from both treatments), that reduced with time showing there was no conversion of fipronil to its sulfide metabolite in the leaves. Reduction of residues of parent fipronil and all the metabolites on the leaves was faster from 5th day onwards and reached below the quantifiable limit of 0.01 mg kg^{-1} at the time of harvest. The residues were subjected to statistical analysis as per Hoskins (1967) to compute the half-life of degradation of fipronil. In grape leaves at recommended dose the residues of fipronil and total fipronil dissipated at the half-life of 9.6 and 13.6 days, respectively and at double dose 18.32 and 20.06 days, respectively. Grape berries were harvested 60 days after the last spray was given and analysed for fipronil and its metabolites. The residues of fipronil, its metabolites, MB 46513, MB 45950 and MB 46136 from treatments at 62.5 and 125 g ha^{-1} were below quantifiable limit of 0.01 mg kg^{-1}. The residue level of fipronil and all its metabolites in soils (under canopy) collected at harvest from both treatments were also below quantifiable limit of 0.01 mg kg^{-1}.

Table 2 Persistence of fipronil residues on grape leaves, grape berries and soil

Days after treatment	Residues recovered* (mg kg^{-1})								
	Untreated control	Application @ 62.5 g ha^{-1}				Application @ 125.0 g ha^{-1}			
		Fipronil	MB 45950	MB 46513	MB 46136	Fipronil	MB 45950	MB 46513	MB 46136
0	BDL	4.120 ± 0.04 (4.805)	0.140 ± 0.003	0.27 ± 0.005	0.275 ± 0.004	6.56 ± 0.101 (7.774)	0.28 ± 0.008	0.462 ± 0.003	0.472 ± 0.006
1	BDL	3.9 ± 0.05 (4.687)	0.128 ± 0.002	0.365 ± 0.006	0.294 ± 0.001	5.90 ± 0.087 (7.62)	0.244 ± 0.004	0.773 ± 0.021	0.703 ± 0.007
3	BDL	3.457 ± 0.107 (4.483)	0.105 ± 0.004	0.493 ± 0.008	0.428 ± 0.009	5.22 ± 0.08 (7.29)	0.205 ± 0.004	1.008 ± 0.035	0.857 ± 0.011
5	BDL	3.1 ± 0.104 (3.918)	0.099 ± 0.001	0.383 ± 0.005	0.336 ± 0.008	4.95 ± 0.108 (6.632)	0.160 ± 0.003	0.865 ± 0.008	0.657 ± 0.010
7	BDL	2.8 ± 0.053 (3.496)	0.089 ± 0.001	0.310 ± 0.007	0.297 ± 0.008	4.62 ± 0.05 (6.032)	0.150 ± 0.002	0.678 ± 0.004	0.584 ± 0.007
12	BDL	2.1 ± 0.053 (2.694)	0.058 ± 0.001	0.262 ± 0.005	0.274 ± 0.005	4.26 ± 0.125 (5.543)	0.128 ± 0.003	0.607 ± 0.014	0.548 ± 0.027
15	BDL	1.62 ± 0.008 (2.162)	0.038 ± 0.000	0.247 ± 0.002	0.257 ± 0.010	3.37 ± 0.026 (4.488)	0.11 ± 0.002	0.497 ± 0.005	0.511 ± 0.008
Leaves at harvest	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Grapes at harvest	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Soil at harvest	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

Figure in the parenthesis is sum of fipronil and it's metabolites

* Average of 3 replicates; BDL < 0.01 mg kg^{-1}

Tingle et al. (2003) reported that fipronil degrades slowly on vegetation and relatively slowly in soil and water, with a half-life ranging between 36 h and 7.3 months depending on substrate and conditions. One of its main degradation products, fipronil desulfinyl is generally more toxic than the parent compound and is very persistent. As per Casida and Hainzl (1996) in a photochemical reaction on the surface of plants fipronil was transformed yielding the desulfinyl photodegradate. In the present study it was found that desulfinyl metabolite (MB 46513) formed about 5.6% of the total residue on the initial day which increased to 11%–13.8% by the 3rd day in the leaves. The sulfone metabolite (MB 46136) increased from about 6% to 9.54%–11.6% from 0(2 h) to 3rd day. These metabolites each formed nearly 11% of the total residues in the leaves by the 15th day. These results show that the above 2 metabolites are more persistent than fipronil. There is also the possibility that fipronil degradation was via transformation to these metabolites. As per the present study, though fipronil is highly persistent in soil it can degrade faster in plants and the temperature and rainfall of tropical climate of India may further reduce the residue deposit. There is no carry over of this insecticide to the next crop as no residues were found in soil at harvest. Therefore insecticide can be used safely on grapes under semi arid climatic conditions of India.

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