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Persistence and Dissipation of Fluopicolide in/on Grape Berries and Soil Under Semi Arid Tropical Climatic Conditions of India

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Abstract Fluopicolide, a relatively new fungicide is used for control of downy mildew of grape. Persistence study of fluopicolide was carried out on grape berries following treatment of the combination product, fluopicolide 4.44% + fosetyl aluminium 66.7% (Profiler 71.14 WG) at the recommended and double the recommended dose of 2.5 and 5.0 kg ha^{-1}. Four treatment sprays were given at 15 day intervals, starting from 1 month after pruning of the vines. Residues of fluopicolide in grape berries were estimated on 0 (1 h), 1, 3, 5, 7, 10, 15, 20, 25, 30 day and at harvest (64 days after the last spray application). The initial residue deposits of fluopicolide on grape berries were 0.58 and 1.32 mg kg^{-1} from treatment at the recommended and double dose, respectively. Dissipation of fluopicolide residues was a slow process. After 30 days of treatment, 0.068 and 0.204 mg kg^{-1} of residues still remained in the berries from treatment at the recommended and double dose, respectively. Fluopicolide residues in grape berries dissipated with the half-life of 10.2 and 12.3 days. Residues in grape berries and soil at harvest were below the quantifiable limit of 0.01 mg kg^{-1} from both the treatments.

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Fluopicolide (2,6-dichloro-*N*-[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]methyl] benzamide), a new fungicide, belongs to the new chemical class, the acylpicolides and is being developed globally in combination with other fungicides for use on a wide variety of crops. Fluopicolide is highly active on the Oomycete organisms responsible for a number of economically important diseases in crops such as grape vine, potato, fruits, vegetables and ornamentals. It has a unique mode of action and shows no cross-resistance with other fungicides (Anonymous 2005). Fluopicolide is effective at low application rates against a wide range of Oomycete (*Phycomycete*) diseases including downy mildews (*Plasmopara, Pseudoperonospara, Peronospora, Bremia*), late blight (*Phytophthora*) and some *Pythium species* (Anonymous 2007). Fluopicolide in combination with propamocarb hydrochloride is superior to metalaxyl and mancozeb in controlling potato late blight (Cooke and Little 2006). Profiler, a WG formulation containing 4.44 g fluopicolide and 66.7 g fosetyl aluminium is very effective in controlling grape downy mildew (*Plasmopara viticola*) at an application rate of 2.25–3.0 kg ha^{-1} (Gouot 2006). Rekanovic et al. (2008) have reported that fluopicolide as a combination formulation is highly effective against *P. viticola*, even when it is applied at long intervals and under high disease pressure. Grape is an important commercial fruit crop of India and it has the highest productivity per unit land area. Annual grape production in the country is estimated to be 1.6 million metric tonnes and area under cultivation about 60 thousand hectares. Approximately 80% of total production, irrespective of variety, is consumed fresh. Downy mildew is a highly destructive disease of grape

vines in all grape growing areas under tropical climatic conditions of India, causing up to 100% losses if the disease is not controlled during favorable weather (Anonymous 2006). Fluopicolide gives effective control of downy mildew and no information is available on its residue persistence in grape berries under semi arid climatic conditions of India. This study was therefore conducted to evaluate residue persistence of fluopicolide on grape berries following treatment of combination product Profiler 71.14 WG.

Materials and Methods

Reference standards of fluopicolide (purity, 99%), and the combination formulation, Profiler 71.14 WG (fluopicolide 4.44% + fosetyl aluminium 66.7%) were obtained from Bayer Crop Science Ltd, India. The chemical structure of fluopicolide is given in Fig. 1. All reagents and solvents used were of analytical grade.

Field experiment was conducted during November 2008- April 2009 at the experimental field of Indian Institute of Horticultural Research, Bangalore, India, on 12 year old grape vines, variety Arka Neelamani. The treatments were untreated control, recommended dose of 2.5 kg ha^{-1} and double the recommended dose of 5.0 kg ha^{-1} of Profiler 71.14 WG (fluopicolide 4.44% + fosetyl aluminium 66.7%). For every treatment ten plants were selected. The spray volume was 1,000 L ha^{-1}. Untreated control vines were sprayed with water. Four treatment sprays were given at 15 day intervals, starting from 1 month after pruning of the vines. After the fourth spray application, grape berries were collected and analysis of residues was carried out on 0 (1 h), 1, 3, 5, 7, 10, 15, 20, 25, 30 day and at harvest (64 days after the last spray). Soil samples from the treated field was collected and analyzed at harvest.

On every sampling day from each vine a grape bunch (approximately 250 g) was collected and pooled together to make a sample size of 2.5 kg. The grape berries were mixed thoroughly and a representative 50 g sample in triplicate were processed for fluopicolide residues. From each plot, soil samples were collected from 3 × 3 grid and 30 cm depth with total 10 sampling sites. From each treatment a total of 5 kg soil samples were collected, mixed thoroughly, air dried and passed through 2 mm sieve. A representative 100 g soil sample in triplicate was processed for residue analysis of fluopicolide.

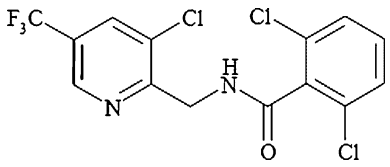

Fig. 1 Chemical structure of fluopicolide

A 50 g portion of grape berries were homogenized, taken in a 250 mL conical flask and 50 mL of pre-heated water (40°C) was added into it. The contents were mixed thoroughly with glass rod and allowed to stand for 30 min. To the flask 100 mL of acetone was added and kept on a mechanical shaker for 2 h. The grape samples were filtered under vacuum through a Buchner funnel. The container and the filter cake were washed twice with 50 mL of acetone. The combined extracts were collected in a 500 mL flask and concentrated under reduced pressure in a rotary vacuum evaporator. To the aqueous fraction 5 g of sodium chloride was added and extracted with 50 mL of mixture of hexane + ethyl acetate (5 + 5, v/v) 3 times. The organic phase was passed through sodium sulphate to remove water content and evaporated to near dryness. Fluopicolide residues were re-dissolved in 5 mL of hexane + ethyl acetate mixture (5 + 5, v/v) for analysis by GLC. Soil samples were processed in a similar manner.

The recovery percent of fluopicolide in grapes berries and soil was estimated at the fortification level of 0.01, 0.05, 0.1, 0.5 and 1.0 mg kg^{-1} before analysis of field samples. The fortified samples (5 replications) were processed as per methodology described above. The results of the recovery study are presented in Table 1.

A Gas Chromatograph (Shimadzu GC-2010) equipped with electron capture detector (ECD) was used for residue analysis of fluopicolide. One micro litre of sample was injected with an auto sampler (AOC-20i auto injector). A capillary column, Agilent DB-5 (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 oven temperature was initially maintained at 140°C hold 5 min and programmed at 4°C min^{-1} to 250°C with hold time of 5 min and 10°C min^{-1} to 280°C with hold time of 9.5 min. Injector and detector temperatures were maintained at 280 and 300°C. Under the above conditions, retention time of

Table 1 Recovery of fluopicolide residues on grape berries and soil at various fortification levels

Fortified concentration (mg kg^{-1})	Mean recovery (%) ± SD*	
	Grape berries	Soil
0.01	87.8 ± 3.56	89.63 ± 2.34
0.05	88.0 ± 2.21	90.38 ± 1.66
0.10	88.5 ± 4.05	90.74 ± 3.47
0.50	89.8 ± 2.44	91.05 ± 1.80
1.00	90.2 ± 1.23	92.12 ± 4.72

* Mean of five replicates analyses

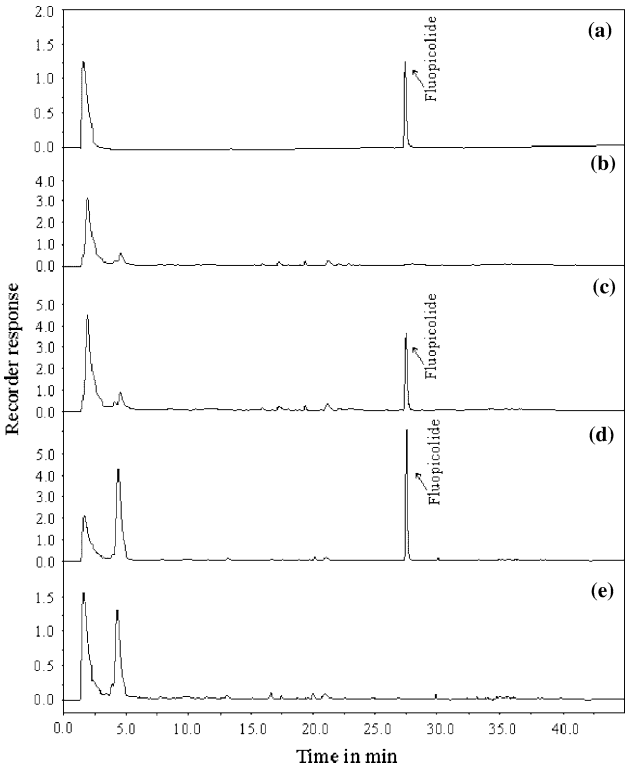

Fig. 2 GLC chromatogram of grape berries; **a** reference standard of fluopicolide; **b** grape untreated control; **c** grape fortified with fluopicolide; **d** field sample of fluopicolide treated grape berries after 20 days; **e** grape berries at harvest

fluopicolide was 27.5 min. The chromatogram of pure standard, fortified, field samples and harvest time grape berries is given in Fig. 2.

Results and Discussion

Recovery study carried out by fortifying grape berries and soil with fluopicolide showed that its recovery in grape berries was in the range of 87.8–90.2% and in soil it was in the range of 89.63–92.12% (Table 1). The limit of quantification of fluopicolide in grape berries and soil using the above method was 0.01 mg kg^{-1}. Analysis of grape samples from field was carried out after the 4th spray over a period of 30 days and in mature berries at harvest. Initial residue deposit of fluopicolide on grape berries was 0.58 and 1.32 mg kg^{-1} from treatment at recommended and double the recommended dose of 2.5 and 5.0 kg ha^{-1}, respectively (Table 2). Residue dissipation of fluopicolide from grape berries was very slow. In the initial stages (up to 5 days) percent dissipation of residues from both the treatments was almost the same. However in the later stages faster dissipation of the residues was observed from treatment at the recommended dose. After 15 days, 64.3% residues had dissipated from treatment at the recommended dose, whereas from double dose treatment 55.7% dissipation had occurred. But after 30 days a very small amount (0.068 mg kg^{-1}) of fluopicolide remained in grape berries from recommended dose of treatment, but from double dose 0.204 mg kg^{-1} still remained (Table 2). The half–life of degradation of fluopicolide was 10.2 and 12.3 days, respectively. At harvest i.e. 64 days after the last spray, fluopicolide residues from both the treatments were below the quantifiable limit of 0.01 mg kg^{-1} in grape berries. Fluopicolide residues estimated in soil collected from grape field at the time of harvest were below the quantifiable limit of 0.01 mg kg^{-1} from both the treatments.

Table 2 Persistence and dissipation of fluopicolide residues in grape berries and soil

Days after treatment	Untreated control	Residues of fluopicolide recovered (mg kg^{-1})							
		Treatment @ 2.5 kg ha^{-1}				Treatment @ 5.0 kg ha^{-1}			
		R_1	R_2	R_3	Mean	R_1	R_2	R_3	Mean
0	BDL	0.587	0.578	0.580	0.582 ± 0.005	1.325	1.322	1.324	1.324 ± 0.001
1	BDL	0.511	0.505	0.510	0.508 ± 0.003 (12.8)	1.131	1.142	1.134	1.136 ± 0.006 (14.4)
3	BDL	0.403	0.431	0.415	0.416 ± 0.014 (28.5)	0.960	0.932	0.942	0.945 ± 0.014 (28.6)
5	BDL	0.357	0.313	0.332	0.334 ± 0.022 (42.6)	0.806	0.750	0.775	0.777 ± 0.028 (41.3)
10	BDL	0.281	0.272	0.275	0.276 ± 0.005 (52.6)	0.705	0.662	0.683	0.683 ± 0.021 (48.4)
15	BDL	0.206	0.210	0.209	0.208 ± 0.002 (64.3)	0.590	0.586	0.584	0.587 ± 0.003 (55.7)
20	BDL	0.161	0.152	0.155	0.156 ± 0.005 (73.2)	0.441	0.410	0.430	0.427 ± 0.016 (67.8)
25	BDL	0.102	0.090	0.095	0.096 ± 0.006 (83.5)	0.302	0.291	0.294	0.296 ± 0.006 (77.7)
30	BDL	0.072	0.066	0.068	0.068 ± 0.003 (88.3)	0.211	0.206	0.195	0.204 ± 0.008 (84.6)
Grape at harvest (64 days)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Soil at harvest	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

Figures in the parenthesis are the percent dissipation of fluopicolide residues

Limit of quantification- 0.01 mg kg^{-1}, BDL <0.01 mg kg^{-1}

Supervised field trials of fluopicolide on grapes have been conducted in some European countries, Canada and USA as per good agricultural practices (GAP). In Northern Europe 3 spray applications of WG formulation of fluopicolide and fosetyl aluminium mixture at the rate of 0.125 kg a.i. ha^{-1} at an interval of 14 days resulted in the residue deposit of 0.21–0.52 mg kg^{-1} after a gap of 28–29 days (Anonymous 2009). Residues of 2 major metabolites were most of the times less than the limit of quantification value of 0.02 mg kg^{-1}. Studies conducted in other part of Europe showed residue levels being in the range of 0.11–0.60 mg kg^{-1} after a period of 28 days (Anonymous 2009). Similar results were obtained in the present study, i.e. after 25 days, 0.096 and 0.296 mg kg^{-1} fluopicolide was recovered from treatment at the recommended and double the recommended dose. Due to unavailability of reference standards of metabolites the possibility of their presence could not be studied. But plant metabolism studies carried out with [^{14}C] fluopicolide showed that fluopicolide was not metabolized to any great extent and the parent compound was the major component of the radioactive residues (87–95%) in grape berries (Anonymous 2009). The present study shows that 4 spray applications of WG formulation of fluopicolide resulted in residue deposit of fluopicolide at 0.58 and 1.32 mg kg^{-1} which remained for a period of 30 days on the berries, but when mature berries were harvested after 64 days, the residues were below the quantifiable limit of 0.01 mg kg^{-1}. Therefore fluopicolide application may result in prolonged control of downy mildew due to its persistent nature, but will result in residue free produces at harvest.

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