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Persistence of Trifloxystrobin and Tebuconazole on Grape Leaves, Grape Berries and Soil

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Abstract Following four foliar applications of Nativo 75 WG (trifloxystrobin 25% + tebuconazole 50%) on grapes @ 175 and 350 g/ha, resulting in active applications of trifloxystrobin @ 43.75 and 87.5 g a.i./ha and that of tebuconazole @ 87.5 and 175 g a.i./ha, the average initial deposits of trifloxystrobin were observed to be 7.76 and 15.53 mg/kg, respectively, at single and double the application rates. These residue levels dissipated to >85% after 10 days of its application at both the dosages. Similarly, the average initial deposits of tebuconazole were observed to be 13.84 and 26.55 mg/kg at single and double the application rate, respectively. These residue levels dissipated to >90% after 10 days of application at both the dosages. The half-life ($t_{1/2}$) periods of trifloxystrobin on grape leaves were observed to be 2.92 and 3.48 days at single and double application rates, respectively, whereas these values were 2.68 and 3.96 days for tebuconazole. Ripe grape berries and soil samples collected at harvest which happened to be 34 days after the last application, did not show the presence of residues of trifloxystrobin and tebuconazole at their determination limit of 0.01 and 0.02 mg/kg, respectively.

Keywords Trifloxystrobin · Tebuconazole · Persistence · Residues · Half-life

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Nativo 75WG is a water dispersible granular formulation containing 25% w/w trifloxystrobin and 50% w/w tebuconazole. It is a broad spectrum systemic fungicides with protectant and curative properties for use on Brussels sprouts, cabbage, broccoli, cauliflower, carrots and leeks for the control of dark leaf spot, light leafy spot, powdery mildews, phoma leaf spot, white blisters, purple blotch etc. (www.BayerCropScience.com).

Several multiresidue methods are available for the determination of residues of different triazoles in various food products such as processed fruits, vegetables, grapes, wines and strawberries (Garland et al. 1999; Sannino 2004; Zambonin et al. 2002) involving intensive sample preparation such as solid phase extraction which is time consuming and labour intensive. The residues of these fungicides are analysed by gas–liquid chromatography by both nitrogen phosphorous detector (NPD) and electron capture detector (ECD), or by techniques such as liquid chromatography-tandem mass spectrometry for confirmation and quantitation (Bernal et al. 1997; Otero et al. 2003; Schermerhorn and Golden 2005; Trosken et al. 2005). The objective of the present study is to give detailed report on the simple and sensitive gas chromatography-nitrogen phosphorous detector (GC-NPD) method developed for the quantitation of trifloxystrobin and tebuconazole residues in grapes, incorporating the validation parameters such as repeatability and reproducibility. Quality parameters such as precision, linearity and detection limits were also

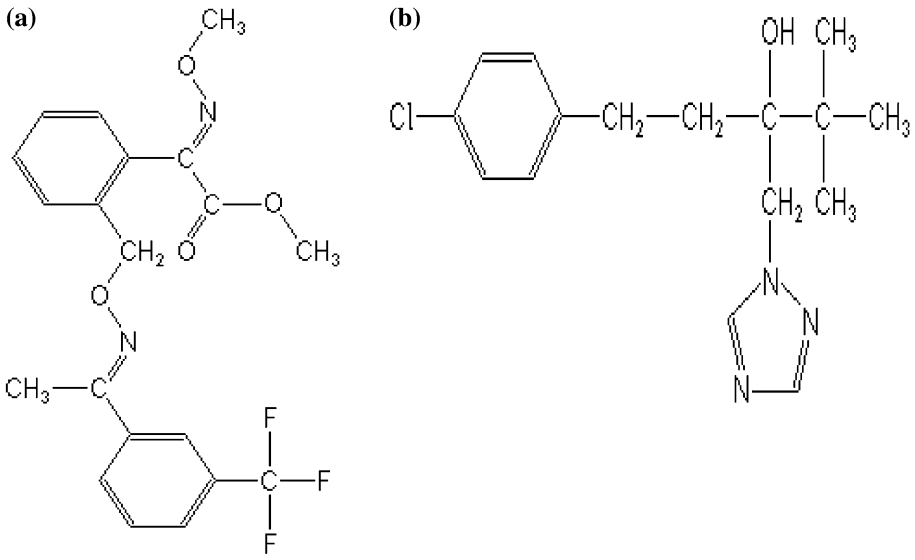

Fig. 1 Chemical structures of (**a**) Trifloxystrobin (**b**) Tebuconazole

evaluated. The aim of present studies is to ensure that the levels of harvest time residues of trifloxystrobin and tebuconazole do not pose any hazard to consumers and are admissible in domestic as well as international trade.

Trifloxystrobin methyl-(E)-methoxyimino-{(E)-α-[1-(α, α, α -trifluoro-m-tolyl) ethylideneaminoxyl]-o-toly} acetate, is a strobilurin fungicide, a new class of substance that are included in the Quinone outside inhibitors (QoI) fungicide groups. The strobilurin fungicides are synthetic active ingredients with similar action to the natural strobilurin A., which is produced by different wood rotting fungi. The fungicidal activity of strobilurins consists in the inhibition of the mitochondrial respiration by binding at the so-called Qo site of cytochrome b, located in the inner mitochondrial membrane of the fungi (Bartlett et al. 2002; Fig. 1).

Tebuconazole (RS)-1-p-chlorophenyl-4, 4-dimethyl-3-(1H-1, 2,4,-triazol-1-ylmethyl)pentan-3-ol, is a broad spectrum fungicide used to control many diseases. (www.bayercropscience.com).

Materials and Methods

The working reference standards of trifloxystrobin and tebuconazole (purity 98.5%) were supplied by M/s Bayer Crop Science Limited, Mumbai. All the solvents used were of laboratory grade. These were redistilled in all glass apparatus and suitability of solvents was ensured by running reagent blank along with actual analysis.

The field experiment was conducted during 2006–2007 on grapes cultivar perlette at PAU Regional Station, Abohar, Punjab, India, and the crop was raised according to recommended agronomic practices (Anonymous 2006). The samples were processed and analysed at Pesticide Residue Analysis Laboratory, department of entomology, PAU, Ludhiana.

Three replications were selected for each treatment i.e. control, recommended dose and double the recommended dose. A total of four applications of Nativo 75 WG (trifloxystrobin 25% + tebuconazole 50%) @175 and 350 g/ha per 1,000 L were made as foliar spray. First application was given 15 days after pruning on 02.04.07 and 2nd application after 10 days of the 1st application on 12.04.07, 3rd application was made at full bloom stage on 23.04.07 and 4th application at berry setting stage on 02.05.07. Control vines were treated with water only.

About 200 g leaves of grapes at different time intervals, 1 kg berries of ripened grapes and 1 kg of soil were collected from each treatment plot at harvest time on 05.06.07. A representative 10 g sample of grape leaves and 50 g of grape berries and soil were processed for residue analysis.

Various researchers have used different methods for extraction and determination of trifloxystrobin and tebuconazole residues depending upon the infrastructure and facilities available (Garland et al. 1999; Giza and Sztwiertnia 2003). The analytical methodology used for grape leaves and grape berries is detailed below.

A representative 10 g sample of grape leaves was chopped and the macerated sample was dipped overnight into 100 mL acetone in an Erlenmeyer flask. The extract was filtered into 1 L separatory funnel, diluted with 600 mL brine solution and partitioned the contents two times into 75 and 75 mL dichloromethane and two times into 75 and 75 mL ethyl acetate. Both dichloromethane and ethyl acetate fractions were combined, dried over anhydrous sodium sulphate and treated with 500 mg activated charcoal powder for about 2–3 h at room temperature. The clear extract so obtained was filtered through Whatman filter paper No. 1, concentrated to near dryness and again added about 20 mL

Table 1 Percent recovery of trifloxystrobin, CGA321113 and tebuconazole on grape leaves, grape berries and soil

Substrate	Fortification level	Recovery (%)[a]		
		Trifloxystrobin	CGA321113	Tebuconazole
Grape leaves	0.01	84.66 ± 5.03	85.00 ± 5.60	86.00 ± 5.00
	0.05	90.00 ± 2.56	91.00 ± 2.00	90.00 ± 3.50
	0.10	91.00 ± 3.10	95.00 ± 3.00	95.00 ± 4.00
Grape berries	0.01	85.00 ± 2.00	89.00 ± 4.56	87.00 ± 3.90
	0.05	88.00 ± 5.60	90.00 ± 5.00	92.00 ± 4.00
	0.10	90.00 ± 3.50	92.00 ± 4.00	95.00 ± 5.20
Soil	0.01	85.00 ± 4.03	88.00 ± 5.03	86.50 ± 2.00
	0.05	90.00 ± 3.80	91.00 ± 2.50	92.00 ± 1.08
	0.10	90.00 ± 4.85	91.00 ± 3.10	95.00 ± 1.00

[a] Mean ± SD of three replicates determination

of acetone was added and concentrated using rotary evaporator at 30°C. Repeated the process to completely evaporate dichloromethane and ethyl acetate and the final volume was reconstituted to about 5 mL using distilled acetone.

The residues of trifloxystrobin and its metabolite CGA321113 and tebuconazole were estimated on GC equipped with Flame Thermoionic Detector (FTD). The operating conditions were; Injector temperature (°C): 300, nitrogen flow rate: 30.0 mL/min, hydrogen flow rate: 3.0 mL/min, Air: 145 mL/min. Under these operating conditions the retention time of metabolite CGA321113, trifloxystrobin and tebuconazole were found to be 3.27, 4.69, and 5.45 min, respectively. Residues were estimated by comparision of peak height/peak area of the standards with that of the unknown or spiked samples run under identical conditions. Half-scale deflection was obtained for 5 ng trifloxystrobin, CGA321113 and tebuconazole and limit of quantification (LOQ) was found to be 0.01 mg/kg for each. Similar methodology was adopted for grape berries and soil. Grape leaves, grape berries and soil samples were spiked with trifloxystrobin at different levels and analyzed as per methodology described above. Percent recovery of trifloxystrobin and its metabolite CGA321113 and tebuconazole in grape leaves, ripe grape berries and soil found to be consistent and more than 80% (Table 1).

Results and Discussion

Trifloxystrobin

Average initial deposits of trifloxystrobin on grape leaves were found to be 7.76 and 15.53 mg/kg, respectively, following the application of trifloxystrobin 25% + tebuconazole 50% (Nativo 75 WG) @43.75 and 87.5 g a.i./ha w.r.t. trifloxystrobin. Residues of trifloxystrobin dissipated below detectable level of 0.01 mg/kg in 15 days at single dosage. Half-life ($t_{1/2}$) values of trifloxystrobin on grape leaves at single and double dosage were observed to be 2.92 and 3.48 days, respectively. Grape berries and soil samples collected after 34 days at harvest time did not reveal the presence of trifloxystrobin and its metabolite CGA 32113 at determination limit of 0.01 mg/kg (Table 2).

These residues levels seem to be quite safe in view of the fact that maximum residue limit (MRL) of trifloxystrobin on grapes is fixed at 2.00 mg/kg (Anonymous 2004).

Likas et al. (2007) had worked on gas chromatographic methods for their determination of trifloxystrobin method and validated on grapes and wine matrices. The residues found in grape samples from field experiments were clearly below the EU established MRL values, thus causing no problems in food safety.

Dissipation of the trifloxystrobin in basidiocarps of Agaricus bisporus was studied in mushroom by Chrysayi et al. (2006). The mushroom samples taken at all three consecutive production flushes following single or split applications of the fungicides were extracted with solvents and the residues were determined by using gas chromatograph equipped with an electron capture detector (GC-ECD). Analysis of trifloxystrobin revealed a quantitative relationship between the application rate (0.8–1.8 g/m^2) and the residue levels of both the parent compound and its acid metabolite. The maximal combined residues of trifloxystrobin and its metabolite were 0.1313 mg/kg. Short- and long-term dietary risk assessment for both fungicides was carried out using consumption data from World Health organization. The potential acute and chronic residues intakes via mushroom consumption were below toxicologically significant indicators.

Table 2 Residues of trifloxystrobin (mg/kg) in grape leaves, grape berries and soil at different times after the application of Nativo 75WG @ 175 and 350 g/ha

Days after application	Trifloxystrobin @ 43.75 g a.i./ha			Trifloxystrobin @ 87.5 g a.i./ha		
	Replicates	Mean ± SD	% Dissipation	Replicates	Mean ± SD	% Dissipation
Before application	BDL	BDL	–	BDL	BDL	–
	BDL			BDL		
	BDL			BDL		
0	8.09	7.76 ± 0.42	–	14.62	15.53 ± 1.91	
	7.28			17.73		
	7.93			14.25		
1	6.68	6.81 ± 0.13	12.24	12.95	12.53 ± 0.75	20.47
	6.81			11.51		
	6.95			12.60		
3	4.15	3.58 ± 0.48	53.86	9.67	8.20 ± 1.31	47.19
	3.35			7.78		
	3.26			7.15		
5	2.45	2.38 ± 0.14	69.32	6.92	6.12 ± 0.73	60.59
	2.48			5.48		
	2.21			5.97		
7	1.95	1.48 ± 0.47	80.92	4.85	4.29 ± 0.73	72.37
	1.00			4.56		
	1.50			3.46		
10	0.91	0.85 ± 0.05	89.04	2.57	2.12 ± 0.39	86.34
	0.85			1.95		
	0.80			1.85		
15	BDL	BDL		0.05	0.05 ± 0.02	99.67
	BDL			0.04		
	BDL			0.08		
Ripe grape berry samples after 34 days	BDL	BDL	–	BDL	BDL	–
	BDL			BDL		
	BDL			BDL		
Soil samples after 34 days	BDL	BDL	–	BDL	BDL	–
	BDL			BDL		
	BDL			BDL		
$T_{1/2}$ (days)	2.92	3.48				

BDL below determination limit of 0.01 mg/kg

Tebuconazole

The average initial deposits of tebuconazole on grape leaves were found to be 13.84 and 26.55 mg/kg, respectively, following four applications at 10 days interval of a Nativo 75 WG (trifloxystrobin 25% + tebuconazole 50%) @ 87.5 and 175 g a.i./ha. Residues of tebuconabzole dissipated below detectable level of 0.02 mg/kg after 15 days at single dosage. Half-life of tebuconazole in grape leaves was observed to be 2.68 and 3.96 days at single and double dosages, respectively. Grape berries and soil samples collected after 34 days at harvest time did not reveal the presence of tebuconazole at the determination limit of 0.02 mg/kg (Table 3).

Garland et al. (1999) had studied the dissipation of tebuconazole in peppermint crops. Three applications of tebuconazole @ 125 and 250 g a.i./ha resulted in detection of residues at 0.26 and 0.80 mg/kg at harvest after 64 days after the last application. Tebuconazole residues were also detected in peppermint oil at range of 0.011 and 0.041 mg/kg.

Karthika and Sachin (2008) determination the hexaconazole residues in black tea. Dissipation of hexaconazole at 200 and 400 mL/ha followed first order

Table 3 Residues of tebuconazole (mg/kg) found in grape leaves, grape berries and soil at different times after the application of Nativo 75WG @ 175 and 350 g/ha

Days after application	Tebuconazole @ 87.5 g a.i./ha			Tebuconazole @ 175 g a.i./ha		
	Replicates	Mean ± SD	% Dissipation	Replicates	Mean ± SD	% Dissipation
Before application	BDL	BDL	–	BDL	BDL	–
	BDL			BDL		
	BDL			BDL		
0	13.84	13.84 ± 1.55	–	22.57	26.55 ± 3.47	
	15.40			28.20		
	12.29			28.90		
1	10.28	9.04 ± 1.22	34.00	19.50	17.56 ± 1.73	33.00
	7.88			17.01		
	8.98			16.17		
3	6.48	6.49 ± 0.47	53.11	13.40	15.04 ± 1.42	43.35
	6.98			15.88		
	6.03			15.86		
5	5.04	4.98 ± 0.10	64.00	7.14	7.22 ± 0.07	72.80
	5.05			7.25		
	4.87			7.29		
7	3.28	3.50 ± 0.68	74.00	5.03	5.19 ± 0.19	80.00
	2.96			5.40		
	4.27			5.15		
10	1.41	1.04 ± 0.32	92.00	2.50	2.55 ± 0.06	90.00
	0.78			2.54		
	0.95			2.62		
15	BDL	BDL		1.95	1.92 ± 0.06	92.00
	BDL			1.98		
	BDL			1.85		
Ripe grape berry samples after 34 days	BDL	BDL	–	BDL	BDL	–
	BDL			BDL		
	BDL			BDL		
Soil samples after 34 days	BDL	BDL	–	BDL	BDL	–
	BDL			BDL		
	BDL			BDL		
$T_{1/2}$ (days)	2.68	3.96				

BDL below determination limit of 0.02 mg/kg

kinetics and residues dissipated below detectibility limit after 14 days in single and double the recommended dosages. The dissipation of tebuconazole in peppers was evaluated by Fenoll et al. (2009). The pepper samples were collected during 6 week period in which two successive applications of tebuconazole were performed. At harvest time residues levels were below detectability limit.

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