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Dissipation Kinetics of Trifloxystrobin and Tebuconazole on Wheat Leaves and Their Harvest Time Residues in Wheat Grains and Soil

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Abstract Following single application of Nativo 75 WG (trifloxystrobin 25 % + tebuconazole 50 %) on wheat crop @ 300 and 600 g ha^{-1}, resulting in active application of trifloxystrobin @ 75.0 and 150.0 g a.i. ha^{-1} and tebuconazole @ 150 and 300 g a.i. ha^{-1}, the average initial deposits of trifloxystrobin on wheat leaves were 5.54 and 8.30 mg kg^{-1}, and that of tebuconazole were 14.66 and 27.94 mg kg^{-1}, respectively. Half-life values for trifloxystrobin were observed to be 2.80 and 2.51 days whereas those for tebuconazole were found to be 2.46 and 1.85 days at recommended and double the recommended dosages, respectively. The residues of trifloxystrobin and tebuconazole dissipated to the extent of >89 % at both the dosages after 7 days. Wheat grain samples at harvest having pre harvest interval of 140 days did not show the presence of trifloxystrobin and tebuconazole at their determination limit of 0.01 mg kg^{-1}.

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

In the realm of food crops in the world, wheat (*Triticum* spp.) occupies the number one position. India is one of the principal wheat producing and consuming countries in the world. It is the second largest producer of wheat in the world, is tipped to harvest a record 84.27 million tones of wheat in 2011 (Anonymous 2011a). In Punjab during 2009–2010 wheat was grown on 35.22 lac hactares with production of 151.69 lac hactares and per hectare yield of 43.07 quintal (Anonymous 2011b). Wheat crop is subjected to many diseases like tan spot (*Pyrenophora tritici-repentis*), spot blotch (*Cochliobolus sativus*), powdery mildew (*Blumeria graminis*), leaf rust (*Puccinia triticina*) and stripe rust (*Puccinia striiformis*) and heavier losses are sustained from these diseases than are in other cereal crops (Wegulo et al. 2009; Duveiller et al. 2007). Fungicides have been established as an essential input in the growing of cereal and it is not possible to grow a profitable crop without the application of fungicides. Despite various limitations there has been tremendous development in fungicide chemistry (McDonald 2006). This was synergised by the development of new fungicide classes with novel modes of action in the 1990s. These include the strobilurins, phenypyrroles, anilinopyrimides, phenoxyquinolines and other compounds that induce defence mechanisms in the plant (Knight et al. 1997).

Trifloxystrobin methyl-(*E*)-methoxyimino-{(*E*)-α,α,α-trifluoro-m-tolyl)ethylidenea minoxyl]-o-toly}acetate, is a broad-spectrum new generation of strobilurin fungicide (Fig. 1) that has high levels of activity against many fungal pathogens within the Ascomycete, Deuteromycete, Basidiomycete, and Oomycete classes (Reuveni 2000). Pests controlled by this active ingredient include grape and cucurbit powdery mildew, apple scab and powdery mildew, peanut leafspot and brown patch of turf grasses (Reuveni 2000; Sudisha et al. 2005; Karaoglanidis and Karadimos 2006). Tebuconazole {(*RS*)-1-*p*-chlorophenyl-4,4-dimethyl-3-(1H-1,2,4-triazol-1-yl methyl) pentan-3-ol} a representative of the triazole fungicide (Fig. 1) is used for disease control on fruits, nut, cereal and vegetable crops word wide. The compound is highly effective in controlling soil-borne and foliar fungal pathogens, and has been

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credited with increasing yields above the levels provided by other widely used fungicides.

Keeping in view all these observations, persistence study of trifloxystrobin and tebuconazole was carried out following application of Nativo 75 WG to understand their behaviour on wheat crop.

Materials and Methods

Trifloxystrobin (99.6 % purity), its metabolite CGA321113 (96.3 % purity) and tebuconazole (97.4 % purity) and its formulation Nativo 75 WG were obtained from M/s Bayer Crop Science Limited (Mumbai, India) (Fig. 1). All the solvents used for the preparation of stock and working standard solutions or in the extraction procedures were obtained from Merck (Darmstadt, Germany). Ethyl acetate, dichloromethane and acetone were of GR grade. Before use these solvents were redistilled and their suitability was ensured by running reagent blanks along with actual analysis. Activated charcoal (LR grade), sodium chloride (ACS reagent grade 99.9 %) and anhydrous sodium sulfate (AR grade) were obtained from sd fine chemicals (Mumbai, India). Certified reference materials of trifloxystrobin, its metabolite CGA321113 and tebuconazole were weighed accurately and stock solutions having concentrations 1 mg kg^{-1} were prepared separately. Working standards were prepared by serial dilution of the stock solution separately.

The studies on residues of trifloxystrobin and tebuconazole on wheat crop (var. PBW 550) were carried out at Punjab Agricultural University experimental farm during November 2010 to April 2011 and the crop was raised according to recommended agronomic practices. Three treatments i.e. untreated control, single dose (300 g a.i. ha^{-1}) and double dose (600 g a.i. ha^{-1}) were selected for carrying out the study. A single foliar application of Nativo 75 WG was done with knapsack sprayer equipped with hollow cone nozzle 50 days after sowing. Untreated control sample was sprayed with water only.

Wheat leaves (250 g) were collected at random from each treatment at 0, 1, 3, 5, 7, 10, 15 and 20 days after the application and brought to laboratory for extraction and estimation of residues. Samples of wheat grains and soil (1 kg each) were collected from the each treatment plot separately at harvest of the crop.

Various researchers have used different methods for extraction and determination of triloxystrobin and tebuconazole residues depending upon the infrastructure and facilities available (Garland et al. 1999; Giza and Sztwiertnia 2003). The analysis of trifloxystrobin, its acid metabolite and tebuconazole was carried out by using method used by Jyot et al. (2010). The whole 250 g wheat leaves were macerated in a high speed blender (Robot Coupe Blixer 6 V.V) and a representative 10 g sample was dipped overnight into 100 mL acetone in an Erlenmeyer flask. The extract was filtered into 1 L separatory funnel was 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 sulfate 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 of acetone 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, trifloxystrobin metabolite CGA321113 and tebuconazole were estimated on GC equipped with Flame Thermoionic detector (FTD). The operating conditions were as follows: Injector temperature (°C): 280; Column temperature (°C): 240; Detector temperature (°C): 300; Nitrogen Flow rate: 30.0 mL min^{-1}, hydrogen flow rate: 3.0 mL min^{-1} and zero air 145 mL min^{-1}. Under these operating conditions the retention time of trifloxystrobin metabolite code CGA321113, trifloxystrobin and tebuconazole were found to be 3.27, 4.69 and 5.45 min, respectively. Residues were estimated by comparison of peak height/peak area of the standards with that of the unknown or spiked samples run under identical conditions. The limit of quantification (LOQ) of trifloxystrobin, trifloxystrobin metabolite code CGA321113 and tebuconazole were found to be 0.01 mg kg^{-1}. Similar methodology was adopted for estimation of residues in soil.

To determine the reliability of the above analytical method, recovery studies were carried out by fortifying control wheat grain, wheat leaves and soil samples at 0.5, 0.25, 0.05 and 0.01 mg kg^{-1}. Recoveries were found in the range of 82–91 % in wheat leaves, 84–89 % in wheat grain

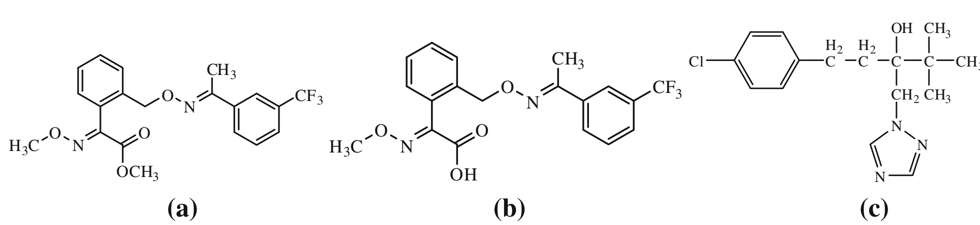

Fig. 1 Chemical structures of **a** trifloxystrobin, **b** its metaboloite CGA321113 and **c** tebuconazole

Table 1 Per cent recovery of trifloxystrobin, trifloxystrobin metabolite code CGA321113 and tebuconazole on wheat leaves and soil at various fortification levels

Substrates	Level of fortification ($mg\ kg^{-1}$)	% Recovery[a]		
		Trifloxystrobin	CGA321113 (trifloxystrobin metabolite)	Tebuconazole
Wheat leaves	0.01	84.56 ± 3.83	89.19 ± 2.00	85.06 ± 1.05
	0.05	86.56 ± 4.83	91.19 ± 3.67	82.56 ± 3.01
	0.25	86.34 ± 2.60	86.58 ± 4.19	82.60 ± 2.44
	0.50	85.11 ± 2.74	89.03 ± 3.25	85.12 ± 3.61
Wheat grains	0.01	86.57 ± 3.07	91.82 ± 2.61	89.85 ± 4.00
	0.05	84.35 ± 5.23	88.23 ± 1.15	85.23 ± 2.22
	0.25	88.23 ± 2.31	82.15 ± 3.20	89.36 ± 1.00
	0.50	84.11 ± 1.31	86.14 ± 2.25	84.23 ± 3.36
Soil	0.01	89.00 ± 2.66	86.82 ± 1.61	90.65 ± 3.55
	0.05	83.57 ± 1.07	91.82 ± 2.61	91.85 ± 2.05
	0.25	89.83 ± 1.37	84.83 ± 4.21	95.74 ± 2.86
	0.50	85.29 ± 3.81	87.20 ± 2.68	89.59 ± 2.82

[a] Mean ± SD of three replicates determinations

Table 2 Residues of trifloxystrobin and CGA ($mg\ kg^{-1}$) found in wheat leaves and soil at different times after the application of Nativo 75 WG @ 300 and 600 g ha^{-1} containing trifloxystrobin @ 75 and 150 g a.i. ha^{-1} respectively

Day after application	Trifloxystrobin @ 75 g a.i. ha^{-1}				Trifloxystrobin @ 150 g a.i. ha^{-1}			
	Trifloxystrobin	CGA321113	Total trifloxystrobin derived residues	% Dissipation	Trifloxystrobin	CGA321113	Total trifloxystrobin derived residues	% Dissipation
Before application	BDL	BDL	BDL	–	BDL	BDL	BDL	–
0 day	5.54[a] ± 0.77	BDL	5.54	–	8.30[a] ± 0.54	BDL	8.30	
1 day	4.06 ± 0.29	BDL	4.06	26.71	6.10 ± 0.72	BDL	6.10	26.51
3 days	2.64 ± 0.42	BDL	2.64	52.35	3.29 ± 0.26	BDL	3.29	60.36
5 days	1.13 ± 0.16	BDL	1.13	79.60	1.86 ± 0.12	0.14 ± 0.01	2.00	75.90
7 days	0.50 ± 0.07	0.06 ± 0.001	0.56	89.89	0.82 ± 0.08	0.08 ± 0.01	0.90	89.16
10 days	0.06 ± 0.004	BDL	0.06	98.92	0.08 ± 0.01	BDL	0.08	99.04
15 days	BDL	BDL	BDL	–	BDL	BDL	BDL	–
Wheat grain at harvest (140 days)	BDL	BDL	BDL	–	BDL	BDL	BDL	–
Soil samples (140 days)	BDL	BDL	BDL	–	BDL	BDL	BDL	–
$T_{1/2}$	2.80				2.51			

BDL below determination limit of 0.01 $mg\ kg^{-1}$

[a] Mean ± SD of three replicates

and 83–95 % in soil. Three replicates for each concentration were analyzed (Table 1).

Results and Discussion

Average initial deposits of trifloxystrobin on wheat leaves were found to be 5.54 and 8.30 $mg\ kg^{-1}$, respectively, following single application of Nativo 75 WG (trifloxystrobin 25 % + tebuconazole 50 %) at the recommended and double the recommended dose of 75 and 150 g a. i. ha^{-1} with respect to trifloxystrobin, respectively. Trifloxystrobin residues dissipated below its determination limit of 0.01 $mg\ kg^{-1}$ after 15 days at both the dosages (Table 2, Fig. 2). The residues of trifloxystrobin dissipated with half-life ($t_{1/2}$) values of 2.80 and 2.51 days, respectively. Wheat

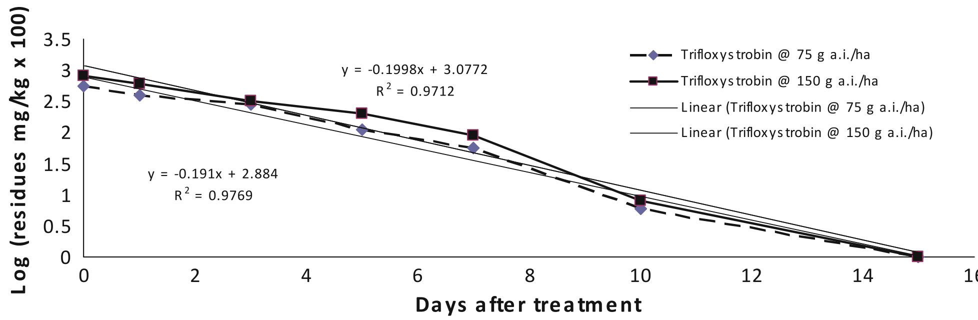

Fig. 2 Semi-logarithmic curve of trifloxystrobin on wheat leaves

Table 3 Residues of tebuconazole (mg kg^{-1}) found in wheat plant and soil at different times after the application of trifloxystrobin 25 % + tebuconazole 50 % (Nativo 75 WG) @ 300 g and 600 g ha^{-1} containing tebuconazole @ 150 and 300 g a.i. ha^{-1}, respectively

Days after application	Tebuconazole @ 150 g a.i. ha^{-1}		Tebuconazole @ 300 g a.i. ha^{-1}	
	Mean ± SD	% Dissipation	Mean ± SD	% Dissipation
Before application	BDL	–	BDL	–
0 day	14.66 ± 2.31	–	27.94 ± 1.911	–
1 day	10.91 ± 0.59	25.57	15.52 ± 0.73	44.46
3 days	6.57 ± 0.52	55.18	8.75 ± 0.61	68.68
5 days	3.64 ± 0.66	75.17	4.75 ± 0.72	82.98
7 days	1.49 ± 0.23	89.84	2.98 ± 0.064	89.33
10 days	0.06 ± 0.01	99.56	0.15 ± 0.05	99.46
15 days	BDL	–	BDL	–
Wheat grains at harvest (140 days)	BDL	–	BDL	–
Soil samples at harvest (140 days)	BDL	–	BDL	–
$T_{1/2}$ (days)	2.46		1.85	

BDL below determination limit of 0.01 mg kg^{-1}

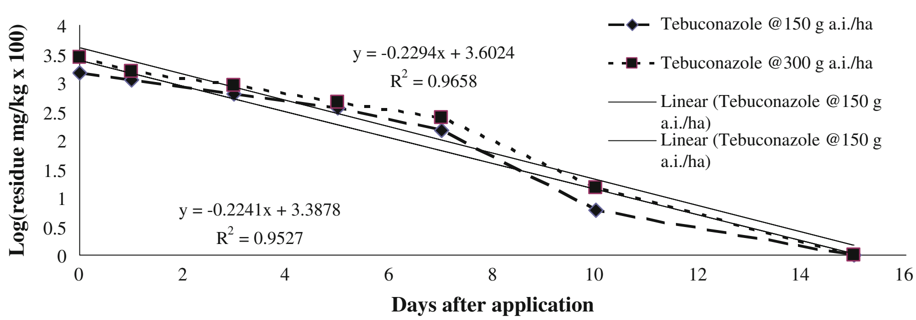

Fig. 3 Semi-logarithmic curve of tebuconazole on wheat leaves

grains and soil samples collected at harvest time from treated plots were found to contain residues of trifloxystrobin below its determination limits of 0.01 mg kg^{-1}.

The results are in agreement with Jyot et al. (2010) who reported the initial deposits of 7.76 and 15.53 mg kg^{-1}, respectively, following the application of trifloxystrobin 25 % + tebuconazole 50 % (Nativo 75 WG) @ 43.75 and 87.5 g a.i.ha^{-1} with respect to trifloxystrobin on grapes. Half-life values of trifloxystrobin on grapes at single and double dosage were observed to be 2.92 and 3.48 days, respectively. Similarly, Mohapatra et al. (2010) determined the initial deposits of 0.305 and 0.875 mgkg^{-1} of trifloxystrobin at recommended dose of 175 g ha^{-1} and double the recommended dose of 350 g ha^{-1} respectively, following application of Nativo 75 WG on grapes.

Sahoo et al. (2012) studied the residues of trifloxystrobin on chili and found the average initial deposits of 0.31 and 0.59 mg kg^{-1}, respectively, following two applications at 10 days interval of Nativo 75 WG @ 250 and 500 g ha^{-1}. The half-life of trifloxystrobin was found to be 1.81 and 1.58 days at recommended and double the recommended dosages, respectively.

The average initial deposits of tebuconazole on wheat leaves were found to be 14.66 and 27.94 mg kg^{-1}, respectively, following single application of Nativo 75 WG (trifloxystrobin 25 % + tebuconazole 50 %) @ 150 and

300 g a.i. ha^{-1} with respect to tebuconazole. Residues of tebuconazole dissipated below its determination limit of 0.01 mg kg^{-1} after 15 days at both the dosages. Half-life of tebuconazole in wheat leaves was observed to be 2.46 and 1.85 days at single and double dosages, respectively (Table 3, Fig. 3).Wheat grains and soil samples collected from treated plots at harvest time did not show the presence of tebuconazole at its determination limit of 0.01 mg kg^{-1}.

Garland et al. (1999) had studied the dissipation of tebuconazole in peppermint crops. Three applications of tebuconazole @ 125 and 250 g a.i. ha^{-1} resulted in detection of residues at 0.26and 0.80 mg kg^{-1} 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^{-1}.

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.

Jyot et al. (2010) reported the initial deposits of 13.84 and 26.55 mg kg^{-1} at recommended dose and double the recommended dose, respectively, following the application of tebuconazole @ 87.5 and 175 g a.i. ha^{-1} on grapes. Similarly, Mohapatra et al. (2010) observed initial deposits of 0.53 and 1.22 mg kg^{-1} respectively, for single and double dose application of Nativo 75 WG.

The average initial deposit of tebuconazole on chili were found to be 0.95 and 1.88 mg kg^{-1} respectively, following two applications at 10 days interval of a Nativo 75 WG (trifloxystrobin 25 % + tebuconazole 50 %) @ 187.5 and 375 g a.i ha^{-1}. The residues dissipated with half-life value of 1.37 and 1.41 days, respectively, for single and double dosages (Sahoo et al. 2012).

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