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Dissipation kinetics of trifloxystrobin and tebuconazole on apple (*Malus domestica*) and soil – A multi location study from north western Himalayan region

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HIGHLIGHTS

- Multi locational persistent data was generated on Nativo 75WG on apple in India.
- The residues of trifloxystrobin and tebuconazole persisted up to 30 d.
- The $t_{1/2}$ of tebuconzole and trifloxystrobin deposits was between 19.38 and 25.99 d.
- Trifloxystrobin metabolite CGA 321113 was found maximum (4.9–12.9%) on 20th day.
- Harvest time residues of Nativo 75WG constituents were BDL in fruits and soil.

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ABSTRACT

A new combinational fungicide formulation trifloxystrobin 25% + tebuconazole 50% (Nativo 75WG), introduced as a part of resistance management strategy, was studied for dissipation behaviour on apple fruits. Nativo 75WG was sprayed twice at the rate of 400 g and 800 g a.i. ha^{-1} equal to trifloxystrobin application rate of 100 and 200 g a.i. ha^{-1} and tebuconazole at application rate of 200 and 400 g a.i. ha^{-1} at four different locations in the Northwest Himalayan region of India. The fruit samples collected at 10 d interval and soil samples taken at harvest time were analyzed after second spray. The residual concentrations of trifloxystrobin, its acid metabolite CGA 321113 and tebuconazole were measured. Residues of both fungicides were determined by using gas chromatograph, Agilent 6890N having electron capture detector. The mean initial deposits of trifloxystrobin at four locations were found to be in the range of 0.333–0.387 mg kg^{-1} and 0.512–0.714 mg kg^{-1} at the application rate of 100 and 200 g a.i. ha^{-1}, and half-life were found between 19.38–24.93 d and 19.84–28.86 d at the respective doses. The Σ-trifloxystrobin and tebuconazole residues were below determination limit in 40 d apple fruits and soil samples. Initial deposits of trifloxystrobin and tebuconazole were below their Codex MRLs at the respective single doses. The half life value of the tebuconazole deposits ranged between 19.38–25.99 d and 19.84–28.86 d at the respective single and double dose. The study thus suggests 1 d pre harvest interval for safe consumption of apple fruit after the application of Nativo 75 WG at single dose.

1. Introduction

Apple is the most important temperate fruit of the Northwest Himalayan region in India. It is a rich source of phytochemicals and has been found to have very strong antioxidant activity which inhibits cancer cell proliferation, decrease lipid oxidation and lower cholesterol (Boyer and Liu, 2004). Early and continuous rains from April onwards do not favour the production of quality fruits besides resulting in high incidence of diseases like *Marssonina* spot *Marssonina coronaria*, leaf spot *Alterneria mali*, powdery mildew *Podospharea leucotricha* and apple scab *Venturia ineaqualis* (Annonymous, 2009) resulting in significant reduction in fruit yield. In recent-past, there has been an increased commercial interest in developing pesticides or fungicides mixtures to improve disease control mechanism by broadening the spectrum of product efficiency as curative, protectant and as robust alternative for resistance management. Nativo 75 WG (50% w/w tebuconazole + 25% w/w trifloxystrobin) a package mix of a sterol-inhibiting fungicide and a strobilurin fungicide has been introduced on apple against scab, rusts and powdery mildew diseases by M/S Bayer CropScience. Trifloxystrobin methyl-(E)-methoxyimino-{(E)-a-[1-(a,a,a-trifluoro-m-tolyl) ethylideneaminoxyl]-o-toly} acetate, is a

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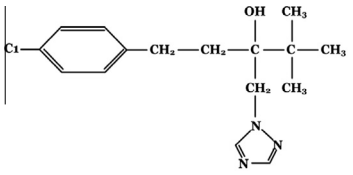
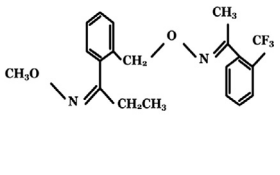
strobilurin fungicide, a new class of substance that is included in the Quinone outside inhibitors (QoIs) fungicide groups. The strobilurin fungicides are synthetic active ingredients with similar action to the natural strobilurin A., which is produced by different wood rotting fungi. The fungicidal activity of strobilurins inhibits the mitochondrial respiration by binding at the Qo site of cytochrome b, located in the inner mitochondrial membrane of the fungi (Bartlett et al., 2002). 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 (Bayer CropScience, 2012).

Dissipation rate of the pesticides is one of the most important parameters in assessing their fate in the environment. The application of Nativo 75 WG near to harvest can leave harmful residues on apple fruits and cause adverse effect on human beings because apple fruits are consumed as raw and even without peeling. Therefore, present study was carried out to study the behaviour of this pesticide on apple fruits and soil following treatment with Nativo 75 WG, a mixture fungicide formulation containing both trifloxystrobin 25% and tebuconazole (50% WG) for registration of this mixture against major diseases of apple in India.



Tebuconazole



Trifloxystrobin

2. Materials and methods

2.1. Field experiment

To study the persistent behaviour of Nativo 75 WG, the experiments were conducted at four different locations of major apple growing areas viz. Solan (1400 m asl), Mashobra (1800 m asl), Matiana (2000 m asl) and Thanedhar (2200 m asl) of Himachal Pradesh, India. Nativo 75 WG was sprayed on fruit bearing apple trees at the rate of 400 g and 800 g ha^{-1}. This application rate becomes to be 100 and 200 g a.i. ha^{-1} of trifloxystrobin and 200 and 400 g a.i. ha^{-1} of tebuconazole. Each treatment was replicated thrice and three apple trees were considered as one replication. A total of two sprays were given i.e. first at 1 month before pre harvest and the second at pre harvest stage. After the second spray, one kilogram fruits were collected randomly from each replication at 0, 10, 20, 30 and 40 d intervals. The fruits were homogenized and analyzed for respective fungicides. Soil samples (1 kg each) were also collected from the trees basin at a depth of 10–15 cm after 40 d of the last treatment. The soil samples were air dried in shade, sieved and analyzed for residues. Meteorological conditions viz; temperature (°C), relative humidity (%) and rainfall (mm) were recorded during the experiment period i.e. from first spray till completion of experiments (Table 1).

Table 1
Meteorological conditions of the experimental locations.

Location	Temperature (°C)		Relative humidity (%)	Rainfall (mm)
	Minimum	Maximum		
Solan	19.83	30.79	77.45	192.30
Mashobra	15.56	22.70	84.33	274.30
Matiana	14.74	20.45	85.72	268.40
Thanedhar	12.52	18.82	86.88	270.90

2.2. Extraction and clean up

2.2.1. Trifloxystrobin and its metabolite

Homogenized apple fruits (25 g) sample was extracted with 100 mL of acetonitrile:water (80:20), filtered through a Buchner funnel under low suction and rinsed with 40 mL of acetonitrile:water (80:20) mixture. The filtrates were pooled and the volume was made up to 200 mL with acetonitrile:water (80:20) mixture. The same method was adopted for processing dried sieved soil sample of 100 g. Pooled filtrate was transferred into a 500 separatory funnel containing 4 mL toluene, 10 mL saturated sodium chloride solution and 20 mL n-hexane. The separatory funnel was shaken for about 1 min and then allowed the phases to separate into three layers: lower-aqueous layer, middle layer-comprised primarily of acetonitrile, water and toluene, top-hexane layer. The lower aqueous layer and top hexane layers were drained out and discarded. Middle layer containing acetonitrile, water and toluene was again partitioned second time for about 1 min. The phases were allowed to separate into two layers. Top hexane layer was discarded and bottom (acetonitrile + toluene + water) layer was drained into a 500 mL round bottom flask. The acetonitrile–toluene was completely evaporated from solution using a vacuum rotary evaporator at 40 °C under reduced pressure. The aqueous phase (3–7 mL) containing residues was transferred into 100 mL separating funnel containing 7 mL of 0.085% phosphoric acid, 1 mL of saturated sodium chloride. It was partitioned with 2×10 mL of tertiary butyl methyl ether (TBME) by shaking for 1 min. Both the TBME fractions were pooled, passed through the layer of sodium sulphate and evaporated to dryness in a rotary evaporator. Residues were dissolved in 5 mL toluene and 1 μL was injected into gas chromatograph.

2.2.2. Tebuconazole

Homogenized 25 g apple fruit sample was extracted with 400 mL acetone:water mixture (3:1) and filtered through Buchner funnel having 15 g filter aid (Celite 545) over Whatman filter paper no. 1. The filtrate was transferred to separatory funnel containing 25 g sodium chloride and extracted the aqueous material with 100 mL dichloromethane by shaking rigorously for 2 min. Organic phase was dried over sodium sulphate and evaporated to dryness in rotary evaporator at 40 °C. The residues were dissolved in 10 mL n-hexane:ethyl acetate (1:4 v/v) mixture. Similar method was adopted for processing 100 g soil sample.

Table 2
Recovery of trifloxystrobin, metabolite CGA 32 1113 and tebuconazole in fruits and soil.

Fungicides	Fortification level, $n = 3$ (mg kg^{-1})	Mean recovery (%)		Relative standard deviation (%RSD)	
		Fruits	Soil	Fruits	Soil
Trifloxystrobin (metabolite CGA 321113)	0.01	91.73 (88.63)	85.35 (84.63)	1.944994 (3.70334)	2.687401 (3.191924)
	0.025	93.66 (91.85)	86.66 (90.66)	2.048993 (2.310806)	3.209371 (2.746498)
	0.25	96.66 (94.53)	93.73 (88.00)	3.108978 (3.403368)	3.271499 (2.440617)
	0.50	94.73 (95.40)	87.73 (91.20)	3.632882 (2.135625)	3.215923 (4.008482)
	1.0	97.10 (91.43)	97.13 (92.60)	2.179305 (3.589197)	2.258858 (3.111351)
Tebuconazole	0.05	91.33	92.00	3.244377	3.099618
	0.50	90.33	86.66	2.544751	2.919524
	1.00	89.36	94.70	3.114874	2.760441
	1.50	95.97	91.82	2.363024	3.323396

Figures in parentheses are percent recovery of metabolite CGA 32 1113.