International Journal of Farm Sciences **4(4)** : 153-160, 2015

Persistence of diniconazole on apple, *Malus domestica* fruits and in soil

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ABSTRACT

The present studies were carried out at four different locations of Himachal Pradesh during the year 2005 in order to study the dissipation of diniconazole on apple fruits and in orchard soil below the trees. Diniconazole (Sumi-8, 25 WP) was sprayed on apple trees during fruiting season at two different dosages viz 500 g ai/ha and 1000 g ai/ha. After spray fruits and soil samples were collected to check the presence of residues of diniconazole in them. The apple fruits were collected from all the four locations and initial deposits of diniconazole were found to range between 0.548-0.711 and 1.108-1.336 mg/kg for application rates of 500 and 1000 g ai/ha respectively. The residues dissipated away with half life of 2.487-6.370 days at recommended dose (500 g ai/ha) and 3.763-5.029 days at higher dose (1000 g ai/ha). In the European Union safe waiting period of 18 days has been suggested at the MRL of 0.10 mg/kg for this compound. The relative standard deviation (RSD) ranged between 1.33-4.97 per cent in fruits and 2.30-4.97 per cent in soil samples respectively with the limit of quantification (LOQ) 0.01 mg/kg.

Keywords: Diniconazole; apple; residues; dissipation; waiting period

INTRODUCTION

The pome fruit apple, *Malus domestica* belongs to Malvaceae family and requires long chilling hours to break dormancy and increase the quality of fruits. In India apple is cultivated at high altitudes approximately above 1,200 masl mainly in Himachal Pradesh, Kashmir and higher hills of Uttar Pradesh. Like other fruit crops apple is also vulnerable to damage by various insect pests and diseases. Apple scab, *Venturia inaequalis* is one of the major threats to apple crop in this part of the world. Some other commonly encountered diseases of apple plants are powdery mildew, canker, collar rot and fruit rot. A large number of fungicides having different mode of action have been reported to control apple diseases. M/S

Sumitomo Chemical India Pvt Ltd has introduced Sumi-8, 25 WP having diniconazole {IUPAC name (*E*)-(*RS*)-1-(2,4-dichlorophenyl)-4,4-dimethyl- -(1*H*-1,2,4-triazol-1-yl)pent-1-en-3-ol}as an active ingredient present in it. Diniconazole is a conazole fungicide with systemic, curative and protective action. It inhibits the demethylation of steroids by distrupting ergosterol biosynthes and is effective against powdery mildew, scab, brown rust, septoria and rynchosporium (Menkissoglu-Spiroudi et al 1998, Anon 2006).

Diniconazole is a toxic chemical having acute LD_{50} value of 639 mg/kg for male rats (Anon 1987). Application of diniconazole on the apple trees which are close to harvest is harmful as apple fruit is consumed raw. Solubility of diniconazole in water is 4 mg/kg (Anon1987) which makes it a potential pollutant of ground water if it falls on the soil. In the soil diniconazole residues can harm the soil microflora which ultimately affects the soil fertility. Therefore the present studies were contemplated in order to study the persistence behavior of diniconazole on apple fruits when sprayed close to fruit harvest. The studies were carried out at four different locations in Himachal Pradesh to fulfill the basic requirements for registration of a product with Central Insecticide Board of India and for suggesting safe waiting period for the compound after following good agricultural practice (GAP).

MATERIAL and METHODS

Chemicals

Analytical grade reagents viz acetone, hexane, ethyl acetate, toluene, silica gel, Florisil and sodium sulphate were obtained from M/S Merck Specialties Private Limited, Mumbai, India. Pesticide residue grade charcoal was procured from M/S Fluka Analytical, Sigma-Aldrich Schweiz, Industrie straâe 25, CH-9470 Buchs SG, Schweiz. Diniconazole, technical (99% purity) and formulation (Sumi-8 25 WP) were obtained from M/S Bayer Crop Science Ltd, Mumbai.

Design of experiment

Experiment on the persistence of diniconazole (Sumi-8, 25 WP) was conducted at four different locations namely Solan (private orchard), Mashobra (research station), Matiana (private orchard) and Thanedhar (private orchard) during fruiting season in the year 2005. Trial was laid out in a randomized block design and each treatment was replicated thrice. A single tree represented one replication.

Climatic conditions

During the study period irrespective of location the maximum temperature, minimum temperature, relative humidity and total rainfall ranged between 20-29^{o}C, 15.00-20.30^{o}C, 81.00-89.43 per cent and 368.60-382.80 mm respectively (Table 1).

Table 1. Location-wise meteorological data during experimental year 2005

Location	Temperature (°C)		Relative	Total
	Maximum	Minimum	Humidity (%)	rainfall (mm)
Solan	29.00	20.30	81.00	368.60
Mashobra	22.37	15.37	89.43	382.80
Matiana	22.30	15.30	89.40	382.00
Thanedhar	20.00	15.00	89.43	382.80

Application of fungicide

Fungicide diniconazole (Sumi-8, 25 WP) was sprayed once on the fruit bearing apple trees at recommended dose 500 g ai/ha (RR) and double the recommended dose 1000 g ai/ha (DRR) using foot sprayer equipped with triple action nozzle. It was sprayed at Solan on 30.07.2005 whereas at Mashobra, Matiana and Thanedhar locations trees were sprayed on 08.07.2005. Control was sprayed with water.

Collection of samples

One kilogram fruit sample was collected randomly for analysis from each replication at 0 (2 hours after spray) and after 1, 3, 5, 7, 10, 15, 20, 25 and 30 pf application. Soil samples (1 kg each) were collected from the tree basins on 0 and 10 days after application.

Extraction and cleanup

Method given by Kadenczki et al (1992) was used for analysis of diniconazole residues. One kilogram fruit sample from each replication was chopped in to small pieces and then homogenised in domestic mixer to obtain fine homogeneous pulp. A representative of 5 g homogeneous sub-sample was taken in a mortar and blended with 10 g Florisil to obtain free flowing powder. Free flowing crop Florisil material was placed on sintered glass column and eluted with 50 ml ethyl acetate. Eluant was collected in 500 ml flask through sodium sulphate and evaporated to dryness in the rotary vacuum evaporator at 40°C. Residues were dissolved in 2 ml hexane and loaded on 2 g silica gel column. Silica gel column was eluted with 15 ml of hexane:acetone (9:1) mixture. Eluant was evaporated to dryness in vacuum rotary evaporator, redissolved the residues in 5 ml toluene and injected one µl into gas chromatograph for residue estimation.

Soil samples were analyzed according to the method given by Brar (2003). The samples were dried in air, grounded and sieved through 100 mm mesh. A sub-sample of 15 g dried soil was blended with 0.3 g Florisil, 0.3 g activated charcoal and 1 g anhydrous sodium sulphate. The contents were

thoroughly mixed and loaded on column. Column was eluted with 100 ml of hexane:acetone (9:1). The eluant was evaporated to dryness and further cleanup was done over silica gel in the same way as described above for fruits.

Estimation of residues

Diniconazole residues were quantified by using Gas Chromatograph (Agilent 6890N) equipped with DB-5 Ultra Performance Capillary column (Cross-linked Methyl Silicone, film thickness: 0.33 microns, internal diameter: 0.25 mm, length: 25 metre).

Instrument conditions

Column temperature started at 160°C for 2 minutes then temperature was increased @ 30°C to reach at final temperature of 260°C. Injection port and detector (ECD) were kept at 260°C and 300°C respectively. Iolar nitrogen flow rate was @ 4 ml/min, septa purge @ 2 ml/min and make up gas 25 ml/min. Under these conditions a retention time of 23.41 min was observed for diniconazole.

Method validation

The efficiency of analytical method was estimated by spiking untreated fruit and soil samples with diniconazole at 0.01, 0.05, 0.10 and 0.50 mg/kg levels. The limit of quantification (LOQ) of diniconazole was 0.01 mg/kg. The residue data were subjected to statistical analysis (Hoskins 1961).

RESULTS and DISCUSSION

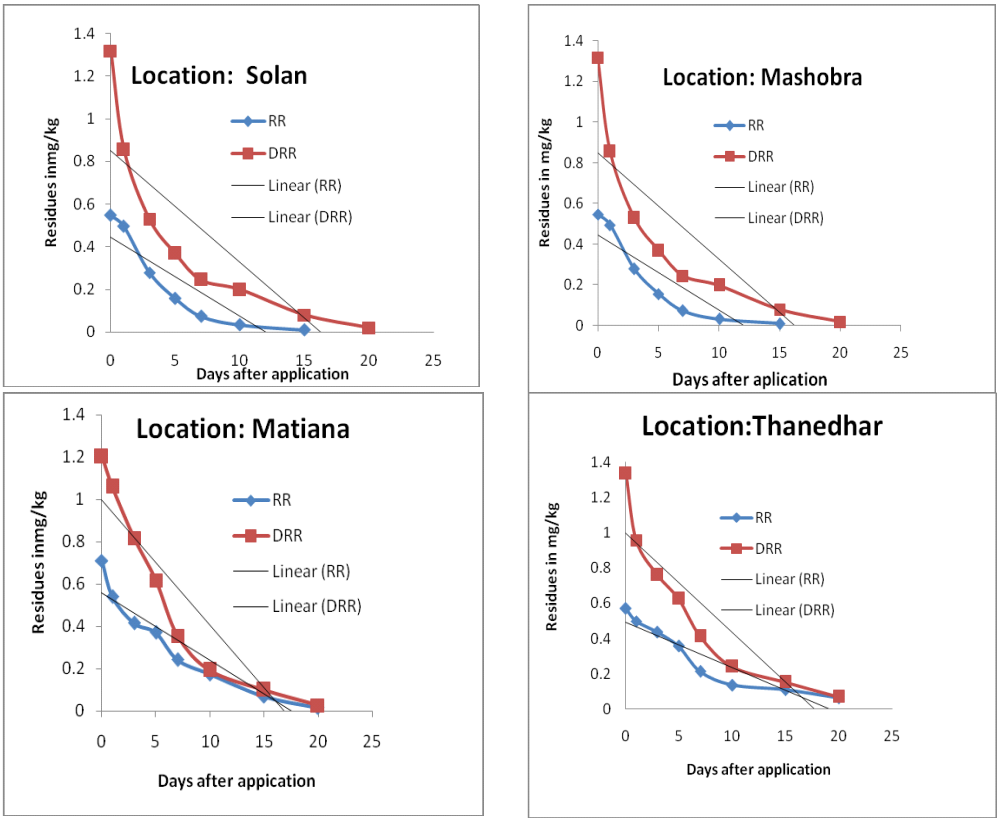
The Table 2 depicts reliability of analytical method tested by spiking of untreated apple fruits and soil samples at different concentrations giving recovery above 90 per cent with relative standard deviation (RSD) of 1.33-4.97 per cent in fruits and 2.30-4.97 per cent in soil samples. The results are in agreement with Hayam Lofty et al (2012) who have observed recovery above 90 per cent using GC-ECD in grapes and Zucchini. The LOQ of this method was found to be 0.01 mg/kg.

The persistence and degradation of the diniconazole was studied at four different locations. Diniconazole was applied at the recommended dose ie 500 g ai/ha and at the double the recommended dose ie 1000 g ai/ha on apple crop. The decrease in level of residues at different locations during days after treatment is presented in Fig 1.

The initial deposits of diniconazole (Sumi-8, 25 WP) on apple fruits were 0.548 and 1.318 mg/kg at Solan which dissipated to 0.010 mg/kg on 15th day and 0.020 mg/kg on 20th day of sampling at single and double dosage respectively. At second location (Mashobra) the initial deposits were 0.711 and 1.108 mg/kg which subsequently dissipated to 0.027 and 0.029 mg/kg on 21st day of sampling at the single and double dosage respectively. The initial deposits 0.710 and 1.203 mg/

Table 2. Recovery of diniconazole from spiked apple fruits and tree basin soil samples

Amount added (mg/kg)	Fruits			Soil		
	Amount recovered (mg/kg)	% RSD	% recovery	Amount recovered (mg/kg)	% RSD	% recovery
0.01	0.009	4.97	90.00	0.009	4.97	90.00
0.05	0.046	4.56	92.00	0.045	3.37	90.67
0.10	0.096	3.13	96.00	0.090	2.30	90.00
0.50	0.488	1.33	97.67	0.471	4.26	94.13



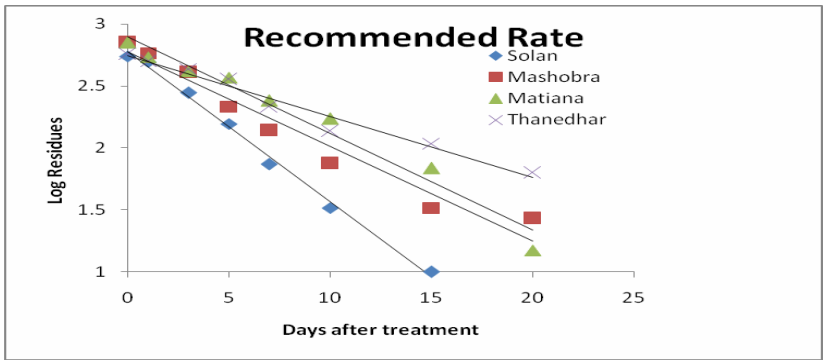
RR= Recommended rate, DRR= Double recommended rate

Fig 1. Residues of diniconazole on apple at different intervals after treatment

Table 3. Pre-harvest intervals and degradation kinetics of diniconazole on apple

Location	Dosage	Regression equation (Y= a+bx)	R	RL_{50}	PHI
Solan	RR	Y= -0.225-0.120x	0.9975	2.5	6.1
	DRR	Y= 0.034-0.082x	0.9905	3.6	13.5
Mashobra	RR	Y= -0.226-0.076x	0.9777	3.9	11.2
	DRR	Y= 0.049-0.077x	0.9973	3.9	13.5
Matiana	RR	Y= -0.105-0.077x	0.9837	3.9	10.9
	DRR	Y= 0.133-0.082x	0.9948	3.7	13.1
Thanedhar	RR	Y= -0.257-0.049x	0.9845	6.1	15.4
	DRR	Y= 0.074-0.062x	0.9955	4.8	18.1

PHI= Pre-harvest interval (calculated at 0.1 mg/kg MRL, Anonymous (2012), RR= Recommended rate, DRR= Double recommended rate, R= Correlation, RL_{50} = Residue half-life



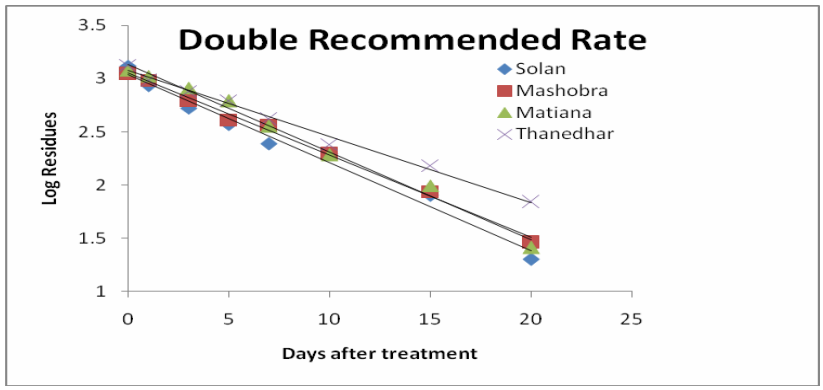


Fig 2. Log residues of diniconazole on apple at different intervals after treatment

kg of diniconazole reduced to 0.015 and 0.026 mg/kg on 21st day of sampling at Matiana when applied @ of 500 g and 1000 g ai/ha. At the fourth location of studies ie Thanedhar the initial deposits recorded on apple fruits were 0.571 and 1.336 mg/kg dissipated to 0.063 and 0.069 mg/kg on 20th day of sampling at single and double dosage respectively. The residue of diniconazole became non-detectable on 30th day. Mohamed Amer et al (2007) have reported diniconazole residues up to 10th day at the recommended dose on tomato and 14 days on green beans. On plotting logarithm of residue concentrations (residues x 1000) against the time lapsed a straight line trend was obtained (Fig 2). The coefficient of correlation reflects decline in residues with the time lapse at both the level of application (Table 2).

The persistence of pesticide is generally expressed in terms of RL_{50} ie time for disappearance of pesticide to 50 per cent of its initial deposit. The RL_{50} values are often obtained by fitting first-order kinetics to observed degradation pattern.

Since diniconazole was not directly applied to soil but its residues were detected in the basin soil on the day of application to the tune of 0.125 and 0.264 mg/kg at Solan, 0.087 and 0.224 mg/kg at Mashobra, 0.102 and 0.284 mg/kg at Matiana and 0.132 and 0.249 mg/kg at Thanedhar at the respective dosage of 500 g and 1000 g ai/ha which became non-detectable on 30th day after the spray .

A perusal of data in Table 3 reveals the degradation kinetics indicating pre-harvest intervals of diniconazole including residue-half life at the respective doses resulting into a safe waiting period of 18 days at an MRL of 0.10 mg/kg set by European Union in 2005 for fruits (Hayam Lofty et al 2012) in Cairo, Egypt. They had observed no residues after 16 days on grapes and zucchini which subsequently reduced below the half-life residue after 6 days on grapes and 2 days in zucchini. In cucumber and pepper grown under green house a safe waiting period of more than 15 days (Mahmoud and Eissa 2007) was recommended before marketing.

ACKNOWLEDGEMENTS

The authors are thankful to M/S Sumitomo Chemical India Pvt Ltd for funding this project.

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Received: 25.6.2014 ***Accepted: 9.9.2014***