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Dissipation Study of Difenoconazole in/on Chili Fruit and Soil in India

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Abstract One field experiment was conducted with Difenoconazole (25% EC) on chili crop during December 2009 to January 2010 at two different locations of West Bengal and Maharashtra. The main objective was to understand the residue and persistence behaviour of fungicide difenoconazole in chili fruit and soil samples. Difenoconazole was applied in chili field at 50 mL and 100 mL/100 liter of water. Its residue was analyzed by using LC–MS/MS and it dissipated in chili fruit and soil following first order kinetics. The half life values of difenoconazole were found in the range 2.15–2.32 days and 4.68–8.09 days for chili fruit and soil, respectively.

Keywords Difenoconazole · Residue · Chili · LC–MS/MS

Chili (*Capsicum annum)* is an essential pillar of the cuisines of India. Chilies are of numerous varieties and their degree of pungency varies. Dry and fresh chilies are used in various Indian sub-continent dishes. Chili is produced all over the world. The largest producer of chili in the world is India (FAOSTAT 2008). India contributes to 25% of the total quantity of chili exported in the world. The world production level has been on an increasing trend and there has been a significant rise in the production level since the late 1990s. The total production has now reached to 7 million tons from 2.5 million tons within 10 years of time (CRNIndia 2010).

Animal and some human studies have indicated that the consumption of chili-containing meals increases energy expenditure and fat oxidation, which may help to reduce obesity and related disorders (Ahuja et al. 2006).

Difenoconazole is a triazole fungicide with broad-spectrum activity against a range of plant pathogens in cereals. It controls numerous pathogens such as *Sepotoria triciti, Sepotoria nordium*, and yellow rust, brown rust and sooty moulds (*Alternaria* spp znd *Cladosporium*). Difenoconazole inhibits ergosterol biosynthesis by inhibiting the fungal enzyme sterol 1-4- α demethylase (anonymous 1994). A literature survey reveals that no work has been published on dissipation of difenoconazole in/on chili fruit and soil in Indian condition. The objective of the present work was to study the dissipation and the fate of difenoconazole residue in/on chili fruit and soil at different location and to develop an efficient residue analytical method. Table 1.

Materials and Methods

A field study in two locations was conducted at Experimental Research Farm, B.C.K.V., Mohanpur, Nadia, West Bengal and All India co-ordinated vegetable Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri-741252, Dist-Ahemednagar, Maharashtra, India. The formulation difenoconazole 25.0% EC was applied plot to plot by knapsack sprayer with flat pan nozzle at 250 mL ha^{-1} (T_1) and at 500 mL ha^{-1} (T_2) [in Randomized Block Designed (RBD) plots] and maintained untreated control (T_3) plots. Three replications were used for each treatment.

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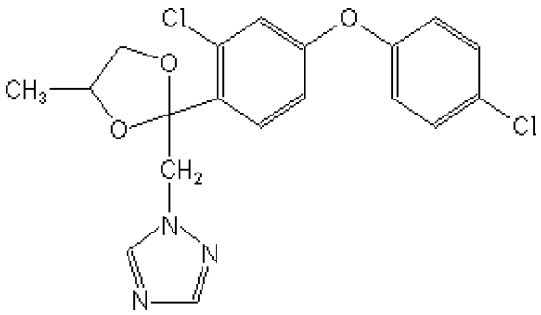
Table 1 Physico-chemical properties of difenoconazole

1.	IUPAC name	3-chloro-4-[(2*RS*,4*RS*;2*RS*,4*SR*)-4-methyl-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether
2.	Empirical formula	$C_{19}H_{17}Cl_2N_3O_3$
3.	Molecular weight	406.2
4.	Melting point	82–83°C
5.	Water solubility	15 mg/L at 25°C
6.	Vapor pressure	3.32×10^{-8} Pa at 25°C
7.	Chemical structure	



Spraying of fungicide was done thrice at 10 days interval for both the locations.

Chili fruits and soil samples were collected at 0, 1, 3, 5, 15 days as well as at harvest time after 3rd application of the fungicide for dissipation study in both the locations. Chili fruits (0.5 kg) and field soil (0.5 kg) samples were collected from 5 to 7 places randomly in each treatment plots replicationwise on each date of sampling. Samples from untreated control plots were also collected separately in the same way. Soil samples were collected from a depth of 10 cm with the help of soil auger.

A representative (10 g) of Chili fruit and soil sample were prepared by quartering technique in the laboratory and taken for final analysis. All the samples were extracted immediately after collection.

A representative chili sample (10 g) from each replication of each treatment was taken in a homogenizer, after cutting into very small pieces. Then these were homogenized for 3 min, afterwards 5 g representative homogenized sample was taken in a 50 mL centrifuge tube and then 10 mL of HPLC grade ethyl acetate was added. The sample was kept undisturbed for 1 h and then 50 mg of activated anhydrous Na_2SO_4 was added to it. The tube was placed over vortex and shaked for 10 min. Then the sample was centrifuged for 20 min at 5,000 rpm. From it 4 mL clear upper layer was transferred into a 10 mL centrifuge tube prefilled with 25 mg florisil and 25 mg PSA. The mixture was then placed in vortex for 2 min and again centrifuged for 15 min at 3,500 rpm. Then 2 mL supernatant liquid was collected and transferred to another centrifuge tube and evaporated to dryness in N_2—evaporator at 25°C. The residue was then reconstituted in 2 mL of [MeOH: H_2O (9:1, v/v) + 5 mM CH_3COONH_4] and it was then filtered through 0.2 μ membrane filter. The sample was then ready for the final analysis in LC–MS/MS.

Soil (5 g) was taken in a 50 mL centrifuge tube & similar method as mentioned in chili fruit sample was followed.

Chromatographic condition

Column	Waters Symmetry C-18, 5 μm2.1 × 100 mm × 100 mm
Eluent	B: 100% {CH_3OH: H_2O (9:1) +5 mM CH_3COONH_4}
Elution	Isocratic (Binary Solvent)
Flow rate	0.3 mL/min
Stop time	3 min
Injection volume	10 μL
Column temp.	25°C ± 0.8°C

Mass Spectrometric Condition

Instrument	Micromass quattro micro API
Ionization mode	ESCi multi-mode
Scan type	MRM
Cappilary voltage (kV)	1.00 kV
Cone voltage (V)	39 V
Extractor (V)	2 V
Source temperature	120°C
Desolvation temperature	350°C
Desolvation gas flow	650.0 (L/h)
Cone (L/h)	25.0 (L/h)
Molecular ion	406.14 amu (used for quantitation)
Mass transition	406.14 → 188.2 amu (for qualitative confirmation) 406.14 → 251.2 amu (for qualitative confirmation) 406.14 → 337.1 amu (for qualitative confirmation)

A linearity check study was carried out with the help of matrix match calibration standard, to know the interference of substrate. In this study a calibration curve was prepared by taking the areas corresponding to different concentrations of matrix match calibration standard. Figure 1.

Recovery studies were carried out in order to establish the reliability of the analytical method and to know the efficiency of extraction and clean up steps employed for the present study, by fortifying the field soil and fruit (chili) samples with different levels of analytical standard solution of difenoconazole. It was carried out by fortifying soil and chili fruit samples separately at the level of 0.01, 0.10 and

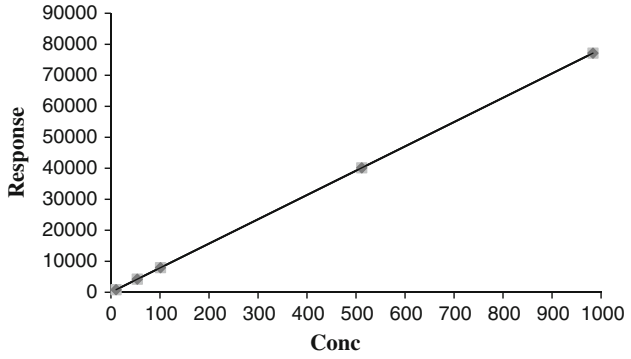


Fig. 1 Callibration curve of difenoconazole

Table 2 Recovery study of difenoconazole in different substrates

Substrate	Amount fortified (mg/kg)	Amount recovered[a] (mg/kg)	Recovery %	Average recovery %
Chili(fruit) sample	0.01	0.0085	85.00	86.67
	0.10	0.0830	83.00	
	0.50	0.4600	92.00	
Field soil	0.01	0.0088	88.00	92.20
	0.10	0.0920	92.00	
	0.50	0.4830	96.60	

[a] Average of three replicates

0.50 mg/kg with the analytical standard solution of difenoconazole and was analyzed following the above mentioned procedure. Results of recovery study are shown in Table 2.

Results and Discussion

Standard calibration curve of difenoconazole was constructed by plotting concentration against peak area. Good linearity was achieved, limit of detection (LOD) and limit of quantification (LOQ) considered when signal to noise ratio of 3:1 and 10:1, respectively. LOD and LOQ were determined as 0.005 and 0.01 mg/kg, respectively. The recovery percentage of difenoconazole from chili and field soil was 86.67% and 92.2%, respectively (Table 2). As the recovery percentage is more than 85% for all the substrates, hence the method can be adopted for residue and dissipation study of difenoconazole in chili samples.

The persistence behavior of difenoconazole in chili has been summarized in Tables 3, 4, 5, 6 in two different locations. A straight line was found when the log of residue was plotted against time and values of coefficient of determination (R^2) at both location in chili samples (fruit and cropped soil) establishing that first order reaction

Table 3 Dissipation of difenoconazole in chili fruit at Maharashtra

Days	Residue in(chili fruit) mg/kg [M* ± SD]	
	T_1 (62.5 ga.i.ha^{-1})	T_2((125 ga.i.ha^{-1})
0	0.09 ± 0.00	0.23 ± 0.00
1	0.07 ± 0.00	0.18 ± 0.00
3	0.04 ± 0.00	0.10 ± 0.00
5	0.02 ± 0.00	0.05 ± 0.00
15	BDL	BDL
Regression equation and half life	$y = 1.9747 - 0.1422x$ $T_{1/2} = 2.12$ days	$y = 2.3725 - 0.1307x$ $T_{1/2} = 2.30$ days

BDL below detection limit, *M** mean of three replicates, *SD* standard deviation

Table 4 Dissipation of difenoconazole in chili cropped soil at Maharashtra

Days	Residue in(chili fruit) mg/kg [M* ± SD]	
	T_1 (62.5 ga.i.ha^{-1})	T_2((125 ga.i.ha^{-1})
0	0.26 ± 0.03	0.41 ± 0.03
1	0.17 ± 0.00	0.25 ± 0.00
3	0.15 ± 0.00	0.22 ± 0.00
5	0.11 ± 0.00	0.16 ± 0.00
15	BDL	0.09 ± 0.00
Regression equation and half life	$y = 2.3660 - 0.0643x$ $T_{1/2} = 4.68$ days	$y = 2.4813 - 0.0372x$ $T_{1/2} = 8.09$ days

BDL below detection limit, *M** mean of three replicates, *SD* standard deviation

Table 5 Dissipation of difenoconazole in chili fruit at West Bengal

Days	Residue in(chili fruit) mg/kg [M* ± SD]	
	T_1 (62.5 ga.i.ha^{-1})	T_2((125 ga.i.ha^{-1})
0	0.08 ± 0.00	0.22 ± 0.00
1	0.07 ± 0.00	0.17 ± 0.01
3	0.04 ± 0.00	0.10 ± 0.01
5	0.02 ± 0.00	0.05 ± .00
15	BDL	BDL
Regression equation and half life	$y = 1.9398 - 0.1391x$ $T_{1/2} = 2.16$ days	$y = 2.3644 - 0.1337x$ $T_{1/2} = 2.25$ days

BDL below detection limit, *M** mean of three replicates, *SD* standard deviation

kinetics was involved in the dissipation process. Initial concentrations of difenoconazole residues in chili fruit and soil samples were found to be 0.23 and 0.41 mg/kg for double dose (125 g a.i./ha), 0.09 and 0.26 for single dose (62.5 g a.i./ha), respectively at Maharashtra. It was found from the results that the half life value was 2.12–2.30 days

Table 6 Dissipation of difenoconazole in chili cropped soil at West Bengal

Days	Residue in(chili fruit) mg/kg [M* ± SD]	
	T_1 (62.5 ga.i.ha^{-1})	T_2 (125 ga.i.ha^{-1})
0	0.27 ± 0.03	0.39 ± 0.02
1	0.19 ± 0.00	0.25 ± 0.00
3	0.17 ± 0.00	0.21 ± 0.00
5	0.12 ± 0.00	0.15 ± 0.00
15	BDL	0.08 ± 0.00
Regression equation and half life	$y = 2.3945 - 0.0623x$ $T_{1/2} = 4.83$ days	$y = 2.4685 - 0.0389x$ $T_{1/2} = 7.74$ days

BDL below detection limit, *M** mean of three replicates, *SD* standard deviation

4.68–8.09 days for chili fruit and soil, respectively at this location.

Similar type of result was also found in West Bengal climatic condition with half life 2.16–2.25 days and 4.83–7.74 days for chili fruit and soil, respectively. The dissipation patterns of difenoconazole of the present study are in well agreement with the earlier studies conducted in cabbage under Chinese climatic condition (Wang et al. 2008).

All the data regarding residues of difenoconazole in harvest chili and cropped soil samples have been presented in Table 7. But, no residue was detected in harvest chili & soil samples irrespective of any doses and locations. However, present harvest residue data is in well agreement with the earlier studies conducted in grape (Banerjee et al. 2008).

Table 7 Harvest residue of difenoconazole in different substrates

Substrate (harvest)	Treatment	Residues in mg/kg				Dissipation (%)
		R_1	R_2	R_3	Mean ± S.D	
Chili fruit	T_1	BDL	BDL	BDL	–	–
	T_2	BDL	BDL	BDL	–	–
Cropped soil	T_1	BDL	BDL	BDL	–	–
	T_2	BDL	BDL	BDL	–	–

BDL below detectable limit

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