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Harvest Residue Study of Fungicide Tebuconazole EC Formulation in Groundnut and Paddy

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ABSTRACT

A field trial was conducted under West Bengal condition during July 2009 *to October* 2009 *to evaluate the harvest residue of Tebuconazole* (25.9% *EC*) *in paddy at two application rates* (750 *and* 1500 *mL·ha*$^{-1}$). *Another field trial was conducted during August* 2009 *to December* 2009 *to evaluate the harvest residue of the same molecule in groundnut. The quantitative analysis of the fungicide residue was performed using Liquid Chromatography-Mass Spectrometry* (*LC-MS/M S*). *The average recovery was found in between* 86.33% *to* 91.87% *for different substrates of groundnut. In case of paddy the average recovery was ranges in between* 86.40% *to* 90.86% *for different substrates. In all the cases, it was found that the fungicide residues were below the detection limit of the instrument* (<0.01 *ppm*) *irrespective of doses in different substrates of paddy and groundnut*. *Based on these findings, the use of Tebuconazole in paddy and groundnut may be advocated for the control of diseases in paddy and groundnut without any residual toxicity problem*.

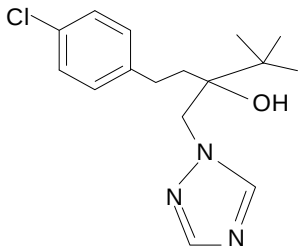
***Keywords*:** *Control*, *Fungicides*, *Pyricularia Oryzae*, *Rice Blast*, *Oryza Sativa L*

1. Introduction

Rice (*Oryza sativa*), one of the three most important food crops in the world, forms the staple diet for 2.7 billion people [1]. It is grown in all the continents except Antarctica, occupying 150 million ha, producing 573 million tonnes paddy with an average productivity of 3.83 tonnes·ha^{-1} [2]. More than 70% grain loss may occur in India as a result of rice blast disease caused by fungus *Pyricularia oryzae* [3].

Similarly groundnut is one of the most important oilseed crop grown in wide range of soil and climate. Leaf spot is one of the most important diseases of groundnut caused by fungus *Sclerotium rolfsii,* causes significant yield losses under Indian climatic condition [4].

Tebuconazole [**IUPAC Name:** (RS)-1-(4-chlorophenyl)-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl)-pentan-3-ol. Ratio (1:1)], a triazole fungicide used as a seed dressing chemical is a systemic fungicide with protective, curative and eradicant action and acts by inhibiting the demethylation of steroids. Used as a spray, it controls numerous pathogens in various crops out of which leaf spot of groundnut, blast disease of paddy are major one [4]. Tebuconazole when applied in the form of fungicide accumulated in surface layers of soil and became toxic to susceptible plants. Also it is rapidly absorbed by vegetative parts of the plants with translocation principally acropetally. The objective of the present work was to study the harvest residue of Tebuconazole EC formulation in groundnut and paddy.



Chemical structure of Tebuconazole.

2. Materials and Methods

A field study was conducted at University Experimental Field, Mohanpur, BCKV, West Bengal, India, during July 2009 to October 2009 on paddy [variety-Khitish]. Another field study was conducted at University Experimental field, Gayeshpur, BCKV, West Bengal, India, during August 2009 to December 2009 on groundnut [variety-JL-24]. The formulation Tebuconazole 25.9% EC was applied with the help of knapsack

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sprayer equipped with WFN 40 nozzle @ 750 mL·ha^{-1} (T_1) and @ 1500 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. The area of every plot was 20 m^2. Spraying of fungicide was done twice at 15 days interval both for paddy and groundnut.

2.1. Collection of Samples and Processing

Different substrates of groundnut (groundnut cropped soil, groundnut plant and groundnut) were collected at the time of harvest following standard sampling procedures. Also different substrates of paddy (paddy plant and paddy cropped soil) were collected at the time of harvest following the same procedure. Groundnut plant, paddy plant, groundnut (0.10 kg) and field soil (0.25 kg) samples were collected from 5 - 7 places randomly from each treatment plots. Samples from untreated control plots were also collected in the same way. Soil samples were collected from a depth of 6″ with the help of soil auger.

3. Residue Analysis

3.1. Extraction and Clean Up of Samples

Plant samples (Paddy straw/Paddy grain/Husk/Groundnut plant):

The samples were blended using Polytron homogenizer. In each case five gram (5 g) of the homogenized sample was taken in a 50 mL centrifuge tube and 10 mL (Ethyl Acetate: Cyclohexane = 9:1) mixture was added and subjected to vortex for 2 min. After that added 5 gm of activated Na_2SO_4, the sample was again vortex for 3 min. Then the sample was centrifuged for 15 min at 10,000 rpm and then 5 ml supernatant liquid was taken in 10 ml centrifuge tube. Afterwards 25 mg florisil & 25 mg PSA was added to it and vortex for 2 min and the sample was again centrifuged for 10 min at 5000 rpm. Then 3 ml supernatant liquid was collected from it and evaporated to dryness in a N_2–Evaporator at 25˚C. The residue was then reconstituted in 3 ml of [MeOH: H_2O (9:1, v/v) + 5 mM CH_3COONH_4] and subsequently filtered through 0.2 µ membrane filter. Now the sample is ready for the final analysis with LC-MS/MS.

Groundnut Oil:

Deshelled groundnut sample (50 g) was grinded in a grinder and was subjected to Soxhlet extraction with 150 mL of hexane for 6 hours. The extracted oil was collected and the rest portion (deoil cake) was kept separately. The hexane extract was concentrated in rotary vacuum evaporator below 40˚C. The oil thus obtained was collected and from it 1 g of oil was weighed and was subjected to extraction. The oil taken was redissolved in 100 mL of hexane and was partitioned thrice (100 + 50 + 50) with acetonitrile. The acetonitrile fraction was collected over anhydrous Na_2SO_4 and the organic phase was evaporated in a rotary vacuum evaporator below 40˚C and was subjected to column clean up. The oil sample thus obtained was cleaned up using silica gel column conditioned with hexane. The sample was applied in the column and kept for 15 min. It was then eluted with 50 mL hexane and discarded. Then 100 mL toluene was passed through the column and the fraction was collected and concentrated in a rotary vacuum evaporator below 40˚C. Finally, it was reconstituted with [MeOH: H_2O (9:1, v/v) + 5mM CH_3COONH_4] which is ready for analysis in LC-MS/MS.

Groundnut Deoil cake:

The deoil cake (10 g) obtained from the oil extraction step was analysed by following same procedure as described for groundnut plant samples.

Soil (Both paddy and groundnut cropped soil):

Five gram (5 g) soil was taken in a 50 mL centrifuge tube & similar method as mentioned in plant samples was followed.

Instrumentatal Parameters:

Chromatographic condition.

Column	Waters Symmetry C-18, 5 µm, 2.1 × 100 mm
Eluent	**A:** 5% {CH_3OH: H_2O (1:9) + 5 mM CH_3COONH_4} **B:** 95% {CH_3OH: H_2O (9:1) + 5 mM CH_3COONH_4}
Elution	Isocratic (Binary Solvent)
Flow rate	0.2 ml/min
Stop time	5 min
Post time	5 min
Injection volume	5 µl
Column temp.	25˚C ± 0.8˚C

Mass Spectrometric condition.

Instrument	Micromass Quattro micro API
Ionization mode	ESCi multi-mode
Scan type	MRM
Capillary voltage (kV)	1.00 kV
Cone voltage (V)	35 V
Extractor (V)	2 V
Source temperature	120˚C
Desolvation temperature	350˚C
Desolvation gas flow	650.0 (L/hr)
Cone (L/hr)	25.0 (L/hr)
Molecular ion	308.14 amu (used for quantation)
Mass transition	308.14 → 69.6 amu (for qualitative confirmation) 308.14 → 125.1 amu (for qualitative confirmation) 308.14 → 150.9 amu (for qualitative confirmation)
LOD	0.005 ppm. or 0.005 µg/mL
LOQ	0.01 ppm. or 0.01 µg/mL (For all substrate)

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3.2. Linearity Check

A calibration curve (**Figure 1**) was made by plotting seven concentrations (0.01 - 1.00 µg/g) of standard Tebuconazole versus absorption. Also, to know the interference of each substrate, matrix match calibration standard for each substrate was prepared. In this study calibration curve was prepared by taking the areas corresponding to different concentrations of matrix match calibration standard, against which final quantification was done.

3.3. Recovery Test

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 samples with different levels of analytical standard solution of Tebuconazole. It was carried out by fortifying different substrates of paddy and groundnut samples at the level of 0.01, 0.10 and 0.50 ppm with the analytical standard solution of Tebuconazole and was analyzed following the procedure. Results of recovery study are shown in **Table 1** and **Table 2.**

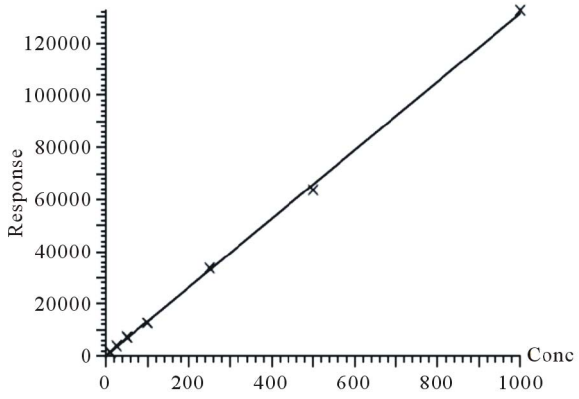

Figure 1. Calibration curve of analytical standard of Tebuconazole.

Table 1. Recovery study of Tebuconazole in different substrates of groundnut.

Substrate	Amount fortified (ppm)	Amount recovered* (ppm)	% Recovery	Average % recovery
Field Soil	0.01	0.009	90.00	91.87
	0.10	0.089	89.00	
	0.50	0.483	96.60	
Groundnut Plant (Haulm)	0.01	0.008	80.00	86.33
	0.10	0.086	86.00	
	0.50	0.465	93.00	
Oil	0.01	0.009	90.00	90.93
	0.10	0.093	93.00	
	0.50	0.449	89.80	
Deoil Cake	0.01	0.009	90.00	89.23
	0.10	0.087	87.00	
	0.50	0.457	91.40	

*Average of three replicates.

Table 2. Recovery study of Tebuconazole in different substrates of paddy.

Substrate	Amount fortified (ppm)	Amount recovered* (ppm)	% Recovery	Average % recovery
Paddy Straw	0.01	0.008	80.00	86.40
	0.10	0.086	86.00	
	0.50	0.466	93.20	
Paddy Grain	0.01	0.009	90.00	90.86
	0.10	0.093	93.00	
	0.50	0.448	89.60	
Husk	0.01	0.009	90.00	89.46
	0.10	0.087	87.00	
	0.50	0.457	91.40	
Field Soil	0.01	0.008	80.00	88.53
	0.10	0.091	91.00	
	0.50	0.473	94.60	

* Average of three replicates.

4. Results and Discussion

4.1. Recovery Study

The recovery percentage of Tebuconazole from different substrates of groundnut and paddy were presented in **Table 1** & **Table 2** respectively. As the recovery percentage is quite high for all the substrates, hence the method can be adopted for harvest residue study of Tebuconazole in different substrate of paddy and groundnut.

4.2. Residues of Tebuconazole in Harvest Samples

All the data regarding residues of Tebuconazole in harvest substrate of paddy and groundnut have been presented in **Table 3** and **Table 4** respectively. In all the cases, it was found that the fungicide residues were below the detection limit of the instrument (<0.01 ppm) irrespective of doses in different substrates of paddy and groundnut. Adiver *et al.* [5,6], stated that tebuconazole effectively control groundnut diseases with no residual toxicity problem. Tirmali *et al.* [7] in 2001 stated same trend in their evaluation study of some new fungicides against rice blast. Moorman *et al.* [8] also reported that, application of Tebuconazole does not possess any residual toxicity problem in soil under vegetable production. Sandra *et al.* [9] and Chuan *et al.* [10] also reported the effectiveness of Tebuconazole against fungal diseases of peppermint crops and apple respectively without possessing any residual toxicity problem. So, it might be stated that Tebuconazole may not pose any residual toxicity problem in paddy and groundnut. Similar observations were also reported earlier about the safety issue of Tebuconazole [11-16]. Based on these findings, the use of Tebuconazole 25.9% EC in paddy and ground-

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Table 3. Harvest residue of Tebuconazole in different substrates of paddy.

Chemical applied	Substrate (Harvest)	Treatment	Residues in ppm.				Dissipation (%)
			R_1	R_2	R_3	Mean ± S.D	
Tebuconazole	Cropped Soil	T_1	BDL	BDL	BDL	-	-
		T_2	BDL	BDL	BDL	-	-
	Straw	T_1	BDL	BDL	BDL	-	-
		T_2	BDL	BDL	BDL	-	-
	Husk	T_1	BDL	BDL	BDL	-	-
		T_2	BDL	BDL	BDL	-	-
	Grain	T_1	BDL	BDL	BDL	-	-
		T_2	BDL	BDL	BDL	-	-

BDL: Below detection limit (<0.01 ppm).

Table 4. Harvest residue of Tebuconazole in different substrates of groundnut.

Chemical applied	Substrate (Harvest)	Treatment	Residues in ppm.				Dissipation (%)
			R_1	R_2	R_3	Mean ± S.D	
Tebuconazole	Cropped Soil	T_1	BDL	BDL	BDL	-	-
		T_2	BDL	BDL	BDL	-	-
	Groundnut Plant	T_1	BDL	BDL	BDL	-	-
		T_2	BDL	BDL	BDL	-	-
	Oil	T_1	BDL	BDL	BDL	-	-
		T_2	BDL	BDL	BDL	-	-
	Deoil Cake	T_1	BDL	BDL	BDL	-	-
		T_2	BDL	BDL	BDL	-	-

BDL: Below detection limit (<0.01 ppm).

nut in West Bengal may be advocated for the control of fungal diseases in paddy and groundnut.

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