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Dissipation and Bioefficacy Studies of Myclobutanil in Chilli under Tarai Region of Uttarakhand, India

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A field study was conducted in Kharif season in Tarai region of Uttarakhand, India from April to August 2015 to determine the efficacy, dissipation and harvest time residues of myclobutanil in chilli using GC-ECD. It was applied at two treatment doses at the rate of 40 and 80 g a.i. ha⁻¹ respectively on the crop. The recovery of myclobutanil in chilli and soil ranged between 86.48 to 98.05 percent. The half lives of myclobutanil were 0.22 to 0.41 days at lower and higher doses of application in chilli. Its residues could not be detected either in chilli or chilli cropped soil at harvest time. Thus, myclobutanil does not appear to pose any threat to consumers or the environment.

Keywords: Fungicide, Vegetables, Half life, Residue, Limit of detection, Limit of quantification.

INTRODUCTION

Myclobutanil [2-*p*-chlorophenyl-2-(1*H*-1,2,4-triazol-1-ylmethyl)hexanenitrile], is a systematic fungicide belonging to the conazole (triazole) chemical family. It has a protective, eradivative and curative action and can rapidly translocate upward and then in basipetal direction in plants [1]. The fungicide inhibits steroid demethylation *via* production of an organic compound ergosterol which is vital for fungal cell wall formation of fungal pathogens [2]. It also has high anti-sporulant activity due to which disease spread is checked in plant. Myclobutanil has been registered as an agricultural fungicide [3] for the control of wide variety of pests in crops like vegetables, stone fruits and cereals.

Chilli, which is one of the most important spice of India belongs to family Solanaceae. It is an essential component in every vegetarian and non-vegetarian Indian diet. Chilli is cultivated in almost all the states of India and is one of the important export commodity [4]. Among the different commercially grown varieties of chilli majority are susceptible to diseases like powdery mildew, fruit rot, bacterial blight and die back [5] which result in a considerable loss of yield, quantity and quality of green and red chilli. Myclobutanil serves as a potent fungicide for the control of majority of susceptible diseases in chilli and is therefore a common choice of farmers. In the past many studies on myclobutanil alone or in combination with other pesticides have been reported for control of several pests in fruits and vegetables [6,7]. However, the presence of

pesticide remains in vegetables, fruits and green leaves is of significant concern from both the health point of view of consumers as well as the environment. Hence it is important to evaluate the crops for the presence of pesticide residues before recommending them for consumption. The monitoring of pesticide is important in soil too as it gives an idea of the extent of soil contamination. Hence in the present study bio efficacy field trials were conducted to determine the persistence of myclobutanil in chilli and also the harvest time residues in chilli cropped soil.

EXPERIMENTAL

The technical grade myclobutanil was obtained from M/s Agropack, Gujarat, India. The chemicals were procured from E Merck, India Pvt. Ltd. and the glasswares used were of corning make.

Field experiment: Bio efficacy field trials were conducted from 01-04-2015 to 12-08-2015 on chilli (Pant chilli 1) in randomized block design at Vegetable Research Centre (VRC), G.B. Pant University, Pantnagar with three replications and two treatments (T1 standard or single dose and T2 double dose) including untreated control (T). The crop was sowed in 3 m × 2.5 m plot in April 2015 and the harvesting was done in August 2015. Application of myclobutanil was done with 10 % WP formulation using a spray solution @ 0.04 % g mL⁻¹ and 0.08 % g mL⁻¹, respectively with a knapsack sprayer at vegetative stage on 01 Jun 2015 (1st application), flowering stage on 13 July 2015 (2nd application) and pre-harvest stage on 29 July 2015

TABLE-I
WEEKLY METEOROLOGICAL DATA DURING THE CROPPING SEASON

Month/date	Date with duration and month	Temperature (°C)		Rainfall (mm)	Relative humidity (%)		Rainy days
		Maximum	Minimum		Maximum	Minimum	
April	02-08	29.40	15.8	18.9	89	45	1
	09-15	31.9	16.6	00.0	82	36	0
	16-22	35.3	18.5	00.0	74	35	0
	23-29	33.7	19.2	01.2	65	34	0
	30-6M	36.1	18.3	18.4	70	29	1
May	07-13	37.4	24.5	10.8	69	39	2
	14-20	37.3	22.5	00.0	70	37	0
	21-27	41.3	22.5	00.8	67	31	0
	28-3J	39.7	22.2	Tr.	65	31	0
June	04-10	41.1	24.5	0.00	62	30	0
	11-17	37.3	25.6	0.08	62	37	0
	18-24	34.9	26.5	166.8	73	54	4
	25-1J	31.4	23.8	230.8	90	76	3
July	02-08	32.7	25.7	110.0	87	72	4
	09-15	32.0	25.4	184.6	88	72	5
	16-22	32.5	26.6	9.8	84	72	2
	23-29	33.8	25.9	6.4	83	63	2
	30-5A	31.4	25.7	148.8	87	74	4
August	06-12	30.4	25.4	89.9	91	76	4
	13-19	32.6	26.1	50.6	90	67	3

(last application). Sampling was done at 0,1,3,5,7,10 and 15 days after the last application. For persistence and residue studies the samples of chilli treated with myclobutanil were collected from each plot at different time intervals as given above and extracted for fungicide residues. In soil myclobutanil residues were determined only at harvest from the chilli cropped soil. The weekly meteorological data comprising of temperature, rainfall, relative humidity and rainy days from the first spray to final sampling was also recorded during the experimental period and is presented in Table-1.

Myclobutanil extraction from chilli and soil samples:

Chilli was finely chopped and soil samples were sieved through 100 mesh sieve for residue analysis. The soil and chilli samples were processed for residue analysis by QuEChERS method given by Anastassiades *et al.* [8], with slight modifications. The chopped chilli and sieved soil samples were extracted with hexane in triplicate. 5 g of soil and 1 g of chilli samples were taken in a 15 mL centrifuge tube separately. Then 5 mL of hexane and 5 mL of distilled water were added to it and the contents were vortexed for 2 min. After giving a stand of 10 min, 4 g of anhydrous MgSO_4 and 1 g of NaCl were added. The mixture was again vortexed for 2 min and then centrifuged for 5 min at 3000 rpm. Thereafter 1 g MgSO_4 and 150 mg primary secondary amine (PSA) reagent were added to the aliquot and the mixture was further centrifuged for 5 min. After phase separation, the upper organic layer was taken and filtered through 0.22 μm PTFE (polytetrafluoroethylene) disc filter and then the samples were analyzed using GC-ECD.

Recovery studies: In order to validate and quantify the efficiency of analytical method used in the study, recovery studies were carried out by spiking the samples of crop and soil at three levels of fortification of myclobutanil (0.01, 0.05 and 0.5 mg/kg) and adopting the same extraction procedure as discussed above.

Residue analysis: The residues of myclobutanil were estimated using gas chromatography (GC) (Ceres 100 plus)

system equipped with capillary column and ECD detector with Iris32 GC software. Carrier gas used was nitrogen, Injection volume was 1 μL , Oven, Injector and Detector temperatures were maintained at 270°C, 280 and 300 °C respectively. The retention time of myclobutanil under the above condition was 5.6 min (Fig. 1)

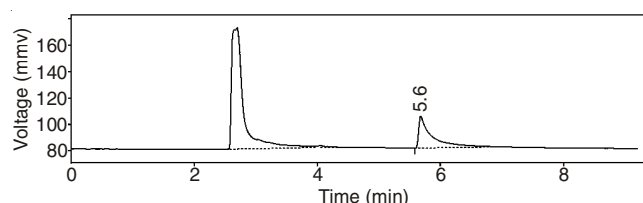


Fig. 1. Chromatogram of standard myclobutanil ($R_t = 5.6$ min)

Linearity check: A linearity check was carried out with the help of analytical standard in a range of 0.005-1 mg kg^{-1} concentration. In order to know the interference of each substrate, matrix match calibration standard for each substrate was prepared. In this study calibration curve (Fig. 2) was plotted by taking the areas corresponding to different concentrations of matrix match calibration standard, against which final quantification was done.

RESULTS AND DISCUSSION

Calibration curve plotted between peak area and different concentrations of analytical standard (Fig. 2) was found to be linear in the range of 0.005-1.00 mg kg^{-1} . The recovery studies carried out in both chilli and soil at three spiked levels (0.01, 0.1 and 0.5 mg kg^{-1}) in triplicate were greater than 90 % (Table-2) indicating that the extraction and quantification method use for myclobutanil is suitable for the study. The LOD and LOQ of myclobutanil in chilli determined in this study on the basis of S/N ratio were found to be 0.005 and 0.01 mg kg^{-1} , respectively.

TABLE-2
RECOVERY OF MYCLOBUTANIL IN CHILLI AND CHILLI CROPPED SOIL

Substrate	Amount fortified (mg/kg)	Average amount recovered $\pm$ SD	Recovery* (%)	Average recovery (%)
Chilli	0.01	0.0090 $\pm$ 0.0020	87.05	92.61
	0.10	0.0930 $\pm$ 0.0120	92.74	
	0.50	0.4900 $\pm$ 0.0330	98.05	
Chilli cropped soil	0.01	0.0086 $\pm$ 0.0020	86.48	92.37
	0.10	0.0930 $\pm$ 0.0017	93.09	
	0.50	0.4860 $\pm$ 0.0043	97.53	

*Each value is mean of three replicate determinants

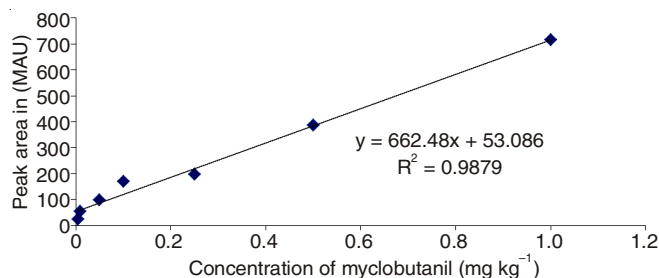


Fig. 2. Calibration curve of myclobutanil

The dissipation pattern of myclobutanil in chilli at single (T1) and double (T2) doses along with untreated control (T) has been summarized in Table-3. The initial deposits on 0 day (2 h after spraying) of myclobutanil in chilli were 0.088 and 0.139 mg kg⁻¹ in T1 and T2 doses respectively. After one day the residue declined to 0.022 mg kg⁻¹ at T1 and to 0.074 mg kg⁻¹ at T2 application rate. On the third day the amount of residue at single application dose (T1) was below detection limit (< 0.005 mg kg⁻¹) whereas at double dose (T2) it was 0.0156 mg kg⁻¹. The fungicide residue progressively dissipated and after 5th day onwards no residues were detected at either of the application doses in chilli. No residues were detected in the control plots and harvest time chilli cropped soil samples too. The dissipation of myclobutanil in chilli followed the first-order kinetics with the half-life values ($t_{1/2}$) determined as 0.22 days for T1 dose and 0.41 days for T2 dose, using equations:

$$C_t = C_0 e^{-kt} \text{ and } t_{1/2} = (\ln 2)/k$$

where C_0 is the initial concentration residue of myclobutanil, C_t is the concentration of myclobutanil at time t , $t_{1/2}$ is the dissipation half life and k is the rate constant.

The samples collected at harvest time from T1 and T2 plots along with untreated control were also analyzed at different time intervals but no residue of myclobutanil was detected at any given time interval (Table-3).

Liu *et al.* [9] reported the half life of myclobutanil ranged between 2.2-3.4 days in lychee. Similarly Wang *et al.* [10] reported that the residues of myclobutanil declined progressively with time in green tobacco leaves but persisted till 21 days. Cui *et al.* [7] examined that the half-life of myclobutanil in onion was 3.4 days but in our studies the half lives of myclobutanil were short probably because of higher temperature and rainfall during the course of the study. The fate of pesticide residues is controlled principally by the primary mechanism of degradation as well as adsorption-desorption characteristics [11]. These aspects are influenced both by the physico-chemical characteristics of the chemical compounds and of the environmental matrix.

Therefore, the fate of a compound in the environment is often predictable, only if adequate knowledge of key properties of the chemical and matrix with which it is interacting are known [12]. Though myclobutanil is considered as a persistent pesticide as it is not degraded significantly by air or sunlight [13] but the dissipation rate of myclobutanil was quite fast in chilli in the Tarai region which can probably be due to high microbial activity in the Tarai soil under humid temperatures. Similar results have also been reported from other studies [14].

Conclusion

Thus, from the residual and persistence study it can be concluded that myclobutanil does not create any residual toxicity problem in chilli and thus it can be considered safe for human consumption after the third day of pesticide application.

TABLE-3
PERSISTENCE OF MYCLOBUTANIL IN CHILLI

Days after herbicide treatment	Residue in mg/kg [M* $\pm$ SD] (% of dissipation)		T
	T1	T2	Untreated control
0	0.088 $\pm$ 0.00125(-)	0.139 $\pm$ 0.001247(-)	BDL
1	0.022 $\pm$ 0.00082(74.96)	0.074 $\pm$ 0.00816(46.72)	BDL
3	BDL	0.0156 $\pm$ 0.0048(78.97)	BDL
5	BDL	BDL	BDL
7	BDL	BDL	BDL
10	BDL	BDL	BDL
Regression equation	$y = -1.386x - 2.430$	$y = -0.736x - 1.930$	-
R ² value	0.999	0.997	-
Correlation coefficient	-0.999	-0.975	-
Half life ($t_{1/2}$)	0.22 days	0.41 days	-
Chilli*	BDL	BDL	BDL
Soil*	BDL	BDL	BDL

Below detectable limit (BDL) < 0.01 ppm, *Harvest time (15 days after fungicide application) samples

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