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Dissipation Kinetics of Metalaxyl in Cucumber

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Abstract Residues of metalaxyl were estimated in cucumber fruits using Gas Liquid Chromatography (GLC) with Nitrogen phosphorus detector (NPD). Following three applications of Ridomil-MZ @ 0.3% and 0.6%, the average initial deposits of metalaxyl were observed to be 0.19 and 0.24 mg kg^{-1}, respectively. The residues dissipated below the limit of quantification (LOQ) of 0.02 mg kg^{-1} after 10 days at both the dosages. The half-life values ($T_{1/2}$) of metalaxyl was worked out to be 3.5 and 3.0 days, respectively at 0.3% and 0.6% concentration. Thus, a waiting period of 1 day was suggested for the safe consumption of metalaxyl treated cucumber.

Keywords Metalaxyl · Residues · Dissipation · Waiting period

Cucumber (*Cucumis sativus* L) is popularly known in India as 'Khira' and 'gherkins' and is extensively grown in tropics, subtropics and milder temperate zones of India mainly used as a salad. The cucumber crop suffers heavy losses due to the attack of downy mildew disease caused by *Pseudoperonospora cubensis* (Berk. and Curt.) Rostow due to the cultivation of susceptible cultivars in the Punjab state and mid hills of Himachal Pradesh. The disease has the potential to destroy the crop within a short period of time causing qualitative and quantitative losses in the crop yield. Therefore use of fungicides is essential to raise economical cucumber crop. Various fungicides have been used for the control of downy mildew of cucumber, muskmelon and luffa. Among the fungicides effective against this disease Ridomil MZ (0.25%) has better eradication action and longer persistence (Gupta et al. 1993; Gupta and Shyam 1998 and Thind 1996). Metalaxyl is a systemic, benzenoid fungicide which acts by inhibiting the RNA synthesis. Excessive use of pesticides for the management of insect pests and diseases in crops is being taken seriously by the public due to their ill effects on the human health. Poisoning due to pesticides among the human beings throughout the world has increased from 500,000 cases per year in 1972 to 2,5000,000 cases per year in 1990 (Levine and Doull 1992). Therefore it is important to ensure that the levels of residues of the pesticides at the time of harvest in the food stuffs do not pose any hazard to the consumers and their levels are below the acceptable limits in domestic as well as in international markets. Keeping in mind the dangers of chemicals, present study was undertaken to know the persistence of metalaxyl in cucumber under subtropical conditions of Punjab, India.

Materials and Methods

The reference standard metalaxyl (98.3% purity) was obtained from Syngenta India Ltd. All the solvents used were of analytical grade. These were redistilled in all glass apparatus and the suitability of solvents was ensured by running reagent blanks along with actual analysis. A stock solution of 1,000 ug mL^{-1} was prepared and was further diluted to obtain concentrations of 100.0, 10.0 and 1.0 ug mL^{-1} in distilled acetone. Cucumber (var. Punjab Naveen) was raised during Kharif 2011, at experimental area, department of Plant Pathology, Punjab Agricultural University, Ludhiana following the recommended agronomic practices. The first application of Ridomil-MZ @ of

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0.3% and 0.6% was done at flowering stage followed by two more applications at 10 days interval. Each treatment was replicated thrice and size of each plot was 7.5 square meters. In control plots only water was sprayed. About 1 kg cucumber fruits of marketable size were collected randomly at 0, 1, 3, 5, 7, 10 and 15 days after the third application of the fungicide. Samples were extracted immediately after sampling.

The extraction and cleanup of cucumber samples for residues of metalaxyl was carried out as per procedure reported by Luke et al. (1975) with slight modifications. A representative 50 gm sample of chopped and macerated cucumber fruits was dipped overnight into 100 mL of acetone in an erlenmayer flask. The extract was filtered through the filter paper into 1 L separatory funnel. The residual material was rinsed with 50 mL of acetone and transferred to the same separatory funnel. The contents of separatory funnel were diluted with 500 mL brine solution and partitioned twice each with 75 mL of Dichloromethane. The lower layer was drained into 500 mL beaker through 1.5″ layer of anhydrous sodium sulfate supported on a pre-washed glass wool in a funnel. The extract was concentrated to about 20 mL under rotary vacuum evaporator. The concentrated extract was treated with 500 mg activated charcoal for 2 h to remove coloured impurities. The solution was filtered through whatman filter paper No. 1 and concentrated to near dryness on a rotary evaporator under vacuum and added 2 mL of acetone for further analysis. Analysis of the metalaxyl residue was carried out on a gas liquid chromatography (GLC) (Perkin Elmer) equipped with Nitrogen Phosphorus detector (NPD). A capillary column Elite 5 with 32 mm i.d. and 30 M length was used. GC operating parameters were as follows: Carrier gas (N_2) flow rate: 24 mL minute^{-1}, air flow: 145 mL minute^{-1}, hydrogen flow: 3 mL minute^{-1}; Temperature: injection port: 250°C, detector: 300°C and column temperature: 180°C for 2 min to 230°C for 5 min (with rate of change of temperature of 15°C min^{-1}). Under these operating conditions the retention time of metalaxyl was found to be 5.3 min.

Results and Discussion

Residues were estimated by comparison of peak height/peak area of the standard with that of the unknown or the spiked samples run under identical conditions. Half-scale deflection was obtained for 5 ng of metalaxyl. The limit of quantification (LOQ) was found to be 0.02 mg Kg^{-1}. To assess the efficiency of the analytical procedure, each method must be validated by an adequate number of controls and recovery values for the samples being analyzed (Leng 1980). Cucumber samples were spiked with metalaxyl at different levels viz. 0.25, 0.5 and 1.0 mg Kg^{-1} and analyzed as per the methodology described above. Percent recovery was found to be consistent and more than 80% as presented in Table 1. The persistence of metalaxyl has generally been expressed in terms of DT_{50} i.e. time for disappearance of pesticides to 50% of its initial deposit. The DT_{50} of metalaxyl was calculated using Hoskins (1961) formula.

The overall results of analysis of cucumber fruits following three applications of Ridomil-MZ at 0.3% and 0.6% concentrations are presented in Table 2. the mean initial deposits of metalaxyl were 0.19 and 0.24 mg Kg^{-1} on the fruits after 3rd application of Ridomil-MZ when applied at 0.3% and 0.6% respectively. These deposits dissipated to 0.13 and 0.15 mg Kg^{-1} after 1 day, respectively, thereby showing a loss of about 32% and 38% following application of Ridomil-MZ @ 0.3% and 0.6%. After 3 days of the last application the metalaxyl dissipated to around 47% and 50%. Both the residues reached below the detectable limit of 0.02 mg Kg^{-1} in 15 days. Half-life ($T_{1/2}$) of metalaxyl calculated as per Hoskins (1961) was 3.5 and 3.0 days, respectively when applied at 0.3% and 0.6% concentrations of Ridomil-MZ. Milgroom and Fry (1988) observed that metalaxyl residues decreased rapidly in the first 2 days of application in potato and thereafter the decrease was quite slow. Singh and Pundhir (2004) observed that only 15% of the metalaxyl residues were observed in potato tubers after 15 days of application.

Persistence of metalaxyl has been studied earlier by Hanumantharaju et al. (2002) on the tomato fruits. It was found that metalaxyl residues dissipated on tomato fruits with a half-life of 5.23–6.95 days, which was higher as compared to those as obtained in the present study. The results obtained in the present study are in agreement with those of Sharma et al. (2006) who reported the half-life period of 2.3 and 3.9 days at 0.2% and 0.4% concentrations of Ridomil-MZ on bitter gourd. They also reported that the residues dissipated to below detectable level in around 20 days. Singh et al. (1998) studied the persistence of metalaxyl in potato tubers. It was found that with double conc. of Ridomil MZ, metalaxyl accumulates in tubers but the level was within prescribed limits. Codex (Anonymous 1993) has prescribed the maximum residues limit (MRL) of 0.5 mg Kg^{-1} for metalaxyl on cucumber. It is found that

Table 1 Recovery of metalaxyl from cucumber fruits

Substrate	Level of fortification (mg Kg^{-1})	Recovery (%) (mean ± SD)[a]
Cucumber fruits	0.25	81.25 ± 1.88
	0.5	82.60 ± 1.56
	1.0	82.12 ± 1.77

[a] Each value is mean ± SD of replicate determinations

Table 2 Residues of metalaxyl (mg Kg^{-1}) on cucumber at different time intervals after the application of Ridomil-MZ @ 0.3% and 0.6% concentrations

Days after application	Metalaxyl @ 0.3%			Metalaxyl @ 0.6%		
	Replicates	Mean ± S.D	% Dissipation	Replicates	Mean ± S.D	% Dissipation
Before application	BDL	BDL	–	BDL	BDL	–
	BDL			BDL		
	BDL			BDL		
0	0.18	0.19 ± 0.02	–	0.22	0.24 ± 0.02	–
	0.21			0.24		
	0.18			0.26		
1	0.12	0.13 ± 0.01	31.58	0.14	0.15 ± 0.01	37.50
	0.13			0.15		
	0.14			0.16		
3	0.09	0.10 ± 0.01	47.37	0.11	0.12 ± 0.02	50.00
	0.09			0.11		
	0.11			0.14		
5	0.07	0.08 ± 0.01	57.89	0.09	0.10 ± 0.01	58.33
	0.08			0.10		
	0.09			0.11		
7	0.06	0.07 ± 0.01	63.16	0.08	0.09 ± 0.01	62.50
	0.07			0.09		
	0.08			0.09		
10	0.03	0.04 ± 0.01	78.95	0.04	0.05 ± 0.01	79.17
	0.04			0.05		
	0.04			0.06		
15	BDL	BDL	–	BDL	BDL	–
	BDL			BDL		
	BDL			BDL		
$T_{1/2}$	3.5 days			3 days		

BDL Below determination limit of 0.02 mg Kg^{-1}

the residues of Ridomil-MZ @ 0.3% and 0.6% concentration were lower than the MRL value at 0 day. Therefore the safe waiting period of 1 day is suggested for the metalaxyl spray on cucumber.

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