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Residue Dynamics of Fenamidone and Mancozeb on Gherkin Under Two Agro Climatic Zones in the State of Karnataka, India

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Abstract Residue dynamics of fenamidone and mancozeb on gherkin was evaluated at two different agro climatic zones i.e. at Bangalore (Zone-1) and Dharwad (Zone-2) in the state of Karnataka, India. Two treatments of the combination formulation (fenamidone 10% + mancozeb 50%) were given at the standard dose 150 + 750 g a.i. ha^{-1} and double dose 300 + 1,500 g a.i. ha^{-1}. Initial residue deposits of fenamidone were 0.467 and 0.474 mg kg^{-1} at Zone-1 and 2, respectively from standard dose treatment. From double dose treatment they were 0.964 and 0.856 mg kg^{-1}, respectively. Fenamidone residues persisted for 15 and 10 days and dissipated with the half-life of 4 and 3 days at Zone-1 and 2, respectively. Mancozeb residue deposits on gherkin were 0.383 and 0.428 mg kg^{-1} from standard dose and 0.727 and 0.626 mg kg^{-1} from double dose treatment at Zone-1 and 2, respectively. Mancozeb residues dissipated with the half-life of 2 and 1 day, respectively. Residues of both fenamidone and mancozeb dissipated faster at Zone-2 compared to Zone-1. The limit of quantification of fenamidone and mancozeb were 0.02 and 0.1 mg kg^{-1}, respectively in both gherkin and soil. Residues of fenamidone and mancozeb in soil collected on the 20th day from the 2 locations were found to be below quantifiable limit of both fungicides.

Keywords Downy mildew · Fenamidone · Gherkin · Mancozeb · Residue

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Gherkin *(Cucumis sativus* L.), is a cucurbitaceous vegetable crop cultivated in India exclusively for exports. Agri export zones (AEZ) have been created for gherkin in Karnataka at Tumkur, Bangalore, Hassan, Kolar, Chitradurga, Dharwad and Bagalkote districts. Gherkin is grown in Karnataka in an area of 39,178 acres and exports during 2004–2005 were 56,581 tonnes (Nabard 2005). Gherkin crop is susceptible to attack by a host of insects and diseases, downy mildew being one of them. Attack by the fungus causes yellow, water soaked lesions or spots on upper surface of leaf and on the corresponding lower surface, grayish or purplish downy growth is seen. Later grayish or purplish colour turns to black because of sporulation. In severe stage, the entire leaf dries quickly. Severely infected vines bear a few small sized fruits not suitable for export (NABARD 2005).

Fenamidone, (S)-5-methyl-2-methylthio-5-phenyl-3-phenylamino-3,5-dihydroimidazol-4-one, provides broad-spectrum control of alternaria leaf spot and downy mildew in cucurbit vegetables, purple blotch in onion, early and late blight in potatoes and tomatoes. (Gary et al. 2010). This fungicide is found to give control of downy mildew of vines (Lacombe et al. 2001) and melon (Gengotti et al. 2009). Mancozeb (Manganese ethylene bis (polymeric) complex with zinc salt) is another fungicide which is widely used for control of downy mildew.

Its formulation, dithane M-45 found to give good control of downy mildew of cucumber (Chaudhury et al. 2009). Mancozeb in combination with metalaxyl was found to be more effective to control downy mildew of cucumber than individually (Somoucha and Cohen, 1984). Fenamidone in combination with other fungicides is also found to be very effective in controlling downy mildew of cucurbits (Colucci and Holmes 2007). Combination formulation of fenamidone 10% + mancozeb 50% has been used for effective control of downy mildew of grapes (Prakash et al. 2007;

Prabhu 2007). This combination formulation has been found to give good control of downy mildew of gherkin. No information is available to our knowledge on the residue persistence of both fenamidone and mancozeb on gherkin. This study was therefore carried out to study the residue dynamics of these two fungicides after application of the combination formulation in two agri export zones i.e. Bangalore (Zone-1) and Dharwad (Zone-2) which are under different agro climatic conditions.

Materials and Methods

Reference standard of fenamidone (99.3%, purity) and combination formulation of fenamidone 10% + mancozeb 50% (Sectin 60 WG) were obtained from M/S Bayer Crop Science Limited, Mumbai, India. All reagents and solvents used were of analytical grade and were purchased locally. Stock solution of fenamidone was prepared (1,000 μg mL^{-1}) with analytical grade acetone and working standards were made by further dilution. For mancozeb estimation analytical grade carbon disulphide (CS_2) was used. Stock solution was prepared with ethanol and diluted further to obtain working standards.

The residue study of fenamidone and mancozeb was carried out at 2 locations i.e. at the experimental farm of Indian Institute of Horticultural Research, Bangalore (IIHR) during March–May, 2010 and at the experimental farm of University of Agricultural Sciences, Dharwad during Nov 2009-January 2010. Combination formulation fenamidone 10% + mancozeb 50% (Sectin 60 WG) spray application was given twice at 2 concentrations, i.e. 150 +750 g a.i. ha^{-1} and 300 + 1,500 g a.i. ha^{-1} of the formulated product. The spray volume was 500 L ha^{-1}. The first spray application was given 38 days after transplanting, and second spray has been given 7 days later. For each treatment 5 plots measuring 5 × 5 M^2 were selected. In both experiment untreated control plots were kept for comparison. Residue analysis of fenamidone and mancozeb was carried out at 0 (2 h), 1, 3, 5, 7, 10, 15 and 20 days after last spray. Gherkin samples from Dharwad were transported in ice boxes to the laboratory at IIHR, Bangalore. The samples were processed immediately on arrival. Soil samples were analysed on the 20th day. From each plot approximately 1 kg soil was collected using a soil augur. Soil from each plot was mixed, air dried and passed through 2 mm sieve.

Extraction of fenamidone residues from gherkin was carried out as per Simonin (2007). A 50 g portion of gherkin representative sample was homogenized with 100 mL acetone + water (9 + 1, vv^{-1}) in a Waring blender and filtered under vacuum through a Buchner funnel. The container and the filter cakes were washed twice with 50 mL acetone + water mixture and the combined extracts were collected in a 500 mL flask. The acetone extract was concentrated under reduced pressure in a rotary vacuum evaporator (Heidolph, Germany). The aqueous extract was transferred to a 1 L separatory funnel and partitioned into 150 mL (50 mL × 3) hexane + ethyl acetate mixture (5 + 5, vv^{-1}) after adding 25 mL saturated sodium chloride solution as described by Abreu et al. (2007). The combined hexane + ethyl acetate extract was dried over anhydrous sodium sulphate, concentrated to dryness and redissolved in 5 mL of acetone for analysis by gas liquid chromatography (GLC). A represantative 100 g soil samples in triplicate was extracted with 150 mL acetone + water (7 + 3, vv^{-1}) and processed in a similar manner.

Analysis of fenamidone residues was carried out by GLC (Varian 3,800) fitted with a thermo ionic specific detector (TSD). A capillary column, Agilent DB-5 (30 m × 0.25 mm id) was used. The carrier gas (ultra pure nitrogen) flow rate was 1 mL min^{-1}, hydrogen flow rate was 3.0 mL min^{-1} and air flow 145 mL min^{-1}. The oven temperature was initially maintained at 140°C with a hold time of 5 min and programmed at 4°C min^{-1} to 250°C with a hold time of 5 min. Injector and detector temperatures were maintained at 280 and 300°C and the injection volume was 1 μL. Under the above conditions, retention time of fenamidone was 16.3 min. The residue data was subjected to statistical analysis according to Hoskins (1961) to compute the residual half-life ($t_{1/2}$) and safe preharvest interval (PHI).

Analysis of mancozeb residues in gherkin was carried out as per Keppel (1971). A 100 g representative sample of gherkin was taken in a 500 mL flask. A mixture of 30 mL concentrated hydrochloric acid, 200 mL distilled water and 5 mL 40% stannous chloride was added to the flask containing the gherkin sample. The flask was refluxed on a heating mantle for 30 min. The CS_2 gas evolved was trapped in ethanolic solution of coloring agent consisting of cupric acetate and diethanolamine. Ethanolic solution of coloring agent was prepared as follows. In a 250 ml volumetric flask 0.012 g of cupric acetate monohydrate was added and dissolved with 25 ml of diethanolamine and the volume was made up to 250 mL with ethanol. The yellow color thus developed was measured spectrophotometrically using a double beam UV—Visible spectrophotometer, Cintra -5 (GBC make) at wavelength of 435 nm. The concentration of CS_2 evolved was calculated from the standard curve prepared with known concentrations of CS_2 solutions.

Recovery study of fenamidone on gherkin and soil was carried out by spiking fenamidone at 0.02, 0.1 and 0.5 mg kg^{-1}. The samples were spiked with 5 replications each and analyzed as per methodology described above before carrying out analysis of field samples. For CS_2 analysis gherkin and soil were spiked at 0.1, 0.5 and

1.0 mg kg^{-1} and analysed spectrophotometrically as described for field sample analysis.

Results and Discussion

Recovery of fenamidone from gherkin was in the range of 83.54%–86.85%, whereas from soil it was in the range of 89.34%–91.32%. The limit of quantification (LOQ) of the method was 0.02 mg kg^{-1} for both gherkin and soil. Recovery pf mancozeb (as CS_2) from gherkin was in the range of 88.53%–89.76% and in soil 90.24%–91.48% (Table 1). The LOQ of the method was 0.1 mg kg^{-1} for both gherkin and soil. The limit of detection of fenamidone and mancozeb was 0.006 and 0.03 ppm, respectively.

Residue evaluation of fenamidone on gherkin from the combination treatment of fenamidone 10% + mancozeb 50% at the recommended and double the recommended dose of 150 + 750 and 300 + 1,500 g a.i. ha^{-1} gave the following results. At zone-1, i.e. IIHR, Bangalore, fenamidone residue deposits immediately after the second application were 0.467 and 0.964 mg kg^{-1} from the recommended and double dose, respectively (Table 2). The residues remained on gherkin for 10 and 15 days, but dissipated at the half-life of 4 days from both treatments. Residue dissipation of fenamidone was faster at Zone-2 (UAS, Dharwad) compared to that of at Zone-1. At Zone-2 the initial residue deposits of fenamidone from the two treatments were 0.474 and 0.856 mg kg^{-1}. Residues remained on gherkin fruits for 7 and 10 days only and dissipated with the half-life of 3 days. Maximum residue limit (MRL) of fenamidone on gherkin is fixed 0.02 mg kg^{-1} by European Food Safety Authority (Anonymous 2010). Based on this value and the results of the residue trials at both locations the pre-harvest interval (PHI) of fenamidone on gherkin was determined to be 15 days from standard dose and 18 days from the double dose treatment.

Mancozeb residue deposits on gherkin from treatments at the recommended and double dose were 0.383 and 0.727 mg kg^{-1} at Zone-1 and 0.474 and 0.646 mg kg^{-1} at Zone-2 (Table 3). From recommended dose of treatment mancozeb residues remained on gherkin for 3 days at both locations. From double dose treatment the residues remained on gherkin fruits for 5 days at Bangalore, but only 3 days at Dharwad. The residues dissipated at the

Table 1 Recovery of fenamidone and mancozeb from gherkin and soil

Fortified concentration (mg/kg)	Mean recovery percent of fenamidone ± SD*		Mean recovery percent of mancozeb (as CS_2) ± SD*	
	Gherkin	Soil	Gherkin	Soil
0.02	83.54 ± 2.54	89.34 ± 3.66	–	–
0.10	84.62 ± 3.62	90.67 ± 4.08	88.53 ± 1.22	90.24 ± 2.68
0.50	86.85 ± 1.58	91.32 ± 2.42	89.04 ± 1.56	91.35 ± 1.34
1.00	–	–	89.76 ± 2.04	91.48 ± 1.06

* Average of five replicate analyses ± SD

Table 2 Residues of fenamidone on gherkin at Bangalore (Zone 1) and Dharwad (Zone 2)

Days after treatment	Untreated control	Residues of fenamidone recovered ± SD (mg kg^{-1})			
		Treatment at 150 + 750 g a.i ha^{-1}		Treatment 300 + 1,500 g a.i ha^{-1}	
		Bangalore	Dharwad	Bangalore	Dharwad
0	ND	0.467 ± 0.017	0.474 ± 0.002	0.964 ± 0.099	0.856 ± 0.052
1	ND	0.291 ± 0.019	0.309 ± 0.011	0.834 ± 0.019	0.677 ± 0.004
3	ND	0.211 ± 0.009	0.197 ± 0.019	0.646 ± 0.015	0.493 ± 0.008
5	ND	0.162 ± 0.004	0.168 ± 0.005	0.460 ± 0.003	0.319 ± 0.008
7	ND	0.123 ± 0.003	0.101 ± 0.005	0.215 ± 0.002	0.151 ± 0.002
10	ND	0.05 ± 0.002	BDL	0.075 ± 0.004	0.050 ± 0.002
15	ND	BDL	BDL	0.041 ± 0.003	BDL
Soil (20th day)	ND	BDL	BDL	BDL	BDL

Limit of Quantification (LOQ)—0.02 mg kg^{-1}

BDL below detection limit, *ND* not detected

* Average of three replicate analyses ± SD

Table 3 Residues of mancozeb on gherkin at Bangalore (Zone 1) and Dharwad (Zone 2)

Days after treatment	Untreated control	Residues of mancozeb recovered ± SD (mg kg^{-1})			
		Treatment at 150 + 750 g a.i. ha^{-1}		Treatment at 300 + 1,500 g a.i. ha^{-1}	
		Bangalore	Dharwad	Bangalore	Dharwad
0	ND	0.383 ± 0.004	0.428 ± 0.016	0.727 ± 0.013	0.646 ± 0.016
1	ND	0.229 ± 0.001	0.261 ± 0.007	0.457 ± 0.015	0.437 ± 0.019
3	ND	0.132 ± 0.006	0.126 ± 0.005	0.250 ± 0.004	0.212 ± 0.006
5	ND	BDL	BDL	0.154 ± 0.006	BDL
7	ND	BDL	BDL	BDL	BDL
Soil (20th day)	ND	BDL	BDL	BDL	BDL

Limit of Quantification (LOQ)—0.1 mg kg^{-1}

BDL below detection limit, *ND* not detected

* Average of three replicate analyses ± SD

half-life of 2 and 1 day, respectively. From standard dose treatment mancozeb residues were below the MRL value of 0.5 mg kg^{-1} immediately after the treatment, while from double dose it reached the MRL within 1 day. Though residues of mancozeb dissipated faster compared to fenamidone residues for the combination product 15 days PHI was recommended. Both fenamidone and mancozeb residues dissipated faster at Dharwad compared to Bangalore. Fenamidone and mancozeb residues in soil at both locations from the 2 treatments were below their respective LOQ 20 days after the last treatment. Mancozeb residues were higher on greenhouse cucumbers (Kaya and Yucel 2002) compared to the present study. The faster degradation of mancozeb in the present study may be due the effect of environmental conditions which is otherwise controlled under greenhouse conditions. During the study period the average maximum temperature was higher at Bangalore whereas relative humidity and rainfall was higher at Dharwad which might have contributed to faster disappearance of the fungicides at that location.

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