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Persistence studies of mancozeb residues in chilli (*Capsicum annum* L.) and soil in Ranga Reddy district of Telangana state, India

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Abstract
An experiment was conducted during *kharif*, 2018 to study the persistence and dissipation of mancozeb residues during fruit formation stage of chilli hybrid (strong 386). Application of mancozeb 75% WP (Dithane M 45) was imposed as foliar spray thrice at 7 days interval at recommended and double the recommended dose of 1500 g *ai*/ha and 3000 g *ai*/ha. Initial residue deposits of mancozeb (as CS_2) observed in green chilli was 0.36 and 1.24 mg/kg which dissipated to less than limit of quantitation of 0.05 mg/kg by 15th day in both the doses respectively. The half life of mancozeb residue was found to be 1.32 and 2.07 days in recommended and double the recommended doses respectively. The residues of mancozeb (based on CS_2) in red chilli and soil collected at harvest were found to be below limit of quantification (LOQ) in both the doses respectively.

Keywords: CS_2, mancozeb, chilli, persistence, pesticide residue analysis

1. Introduction
Chilli (*Capsicum annuum* L.) is an important vegetable crop grown in almost all parts of tropical and subtropical regions of the world, belongs to the family Solanaceae. It is rich in capsaicin, capsanthin, capsorubin, carotenoids, vitamins A, C, E and has antioxidant properties (Wahyuni *et al*., 2011) [15]. It contains about 8.8 g, 5.3 g carbohydrates sugar, 1.9 g protein and 534 micro g beta carotene per 100 g chilli (Panda *et al*., 2010) [9]. Chilli is a universal spice crop of India grown in almost all the states of the country. The increased availability of spice oil and oleoresins of chilli has also increased its consumption in various food preparations. In Telangana, chilli crop is cultivated in area of 11,100 ha with production of181.66 MT whereas in India it is cultivated in area of 3,09,000 ha with production of 3592 MT (Anonymous, 2018). India is the world's largest producer, consumer and exporter of chilli peppers. Chilli suffers from both biotic and abiotic stresses which cause immense losses to the growers.
Mancozeb [[1,2- ethanediylbis] carbamodithioate]] (2-)] manganese mixture with [[1,2-ethanediyl bis [carbamodithioate]] (2-) zinc belongs to the dithiocarbamate grouping of fungicides and more specifically to the class of compounds known as ethylene bis dithiocarbamates (EBDCs). It is a non-systemic fungicide with multi-site, protective action on contact. It is a combination of two other dithiocarbamates; maneb and zineb (Morgan, 1982) [8]. The mixture controls many fungal diseases in a wide range of field crops, fruits, nuts, vegetables, and ornamentals. Chilli is consumed as green chilli as well as red chilli (at harvest), the study on dissipation pattern of commonly used fungicide like mancozeb will help to decide the safe waiting period. Considering the significance of dithiocarbamates in vegetable crop like chilli and soil environment would be of immense value. The present study was carried out to determine the residual persistence and degradation kinetics of mancozeb (as CS_2) in chilli.

2. Materials and Methods
2.1 Test insecticide
Certified reference standards of carbon disulfide with purity of 99 percent and formulation of mancozeb (Dithane M 45 75% WP) were provided by M/s. Dow Agro Sciences India Pvt. Limited.

2.2 Field Experiment
The field trial was carried out at student farm, college of agriculture, PJTSAU, Rajendranagar,

Hyderabad, Telangana, India located at 17º19'19.2" latitude, 78º24'39.2" longitude and at an elevation of 534 m above MSL. The chilli variety, Strong 386 (hybrid chilli) was raised as per the package of practices of PJTSAU, 2018. The weather parameters recorded during the period of study was given in Table 1. The chilli plants were cropped in soil of pH 7.8, EC 0.32, OC 0.42, available nitrogen 210 kg/ha, available phosphorous 44.3 kg/ha and available potassium 351 kg/ha.

2.3 Treatments

Three foliar applications of mancozeb 75 WP was applied by knapsack sprayer fitted with hollow cone nozzle at 7 days interval starting first application at fruit setting stage of green chilli field (56 days after transplanting) with at recommended dose @ 1500 g *a.i.*/ha (T_1); double the recommended dose @ 3000 g *a.i.*/ha (T_2) and untreated control (T_0) ensuring the availability of samples for analysis at different intervals. The plots were laid out in Randomized Block Design (RBD) with three replications having plot size of 30 m^2 (5mx6 m). Spray volume of 500 L ha^{-1} was used.

Table 1: Meteorological parameters recorded during study period

Month & Year	Temperature (ºC)		Relative Humidity (%)		Rainfall (mm)
	Min.	Max.	I	II	
June, 2018	23.8	36.1	86.5	61.1	122.6
July, 2018	23.4	30.2	85.1	68.2	188.2
August, 2018	23.4	29.3	89.1	69.4	316.2
September, 2018	23.2	31.8	87.2	62.3	45.2
October, 2018	23.2	31.8	87.4	62.6	0.0
November, 2018	17.28	30.07	87.7	48.2	0.0
Mean	22.38	31.54	87.1	61.9	112.0

2.4 Sampling

For persistence studies, 500 g of green chilli fruits were collected randomly from each replicate at 0 (after 2 hrs), 1, 3, 5, 7, 10, 15 and 20 days after last application. The sample (1 kg) of red chilli and soil was collected at harvest. The soil samples were collected treatment wise and replication wise following "Z" sampling plan, air dried, ground and sub-sampling was done following quartering method. The collected samples were brought immediately to the laboratory in polythene bags for further residue estimation.

2.5 Chemicals and reagents

Ethyl acetate (HPLC grade), Stannous chloride, Hydrochloric acid, Acetone (AR grade) and Isooctane was procured from Merck Chemicals, Hyderabad. Type-I (18.2 MΩ) ultrapure water was prepared in the laboratory by Elga pure lab water purifier (High Wycombe, UK). The LCMS grade methanol was procured from JT Baker, PA, USA. The suitability of the solvents and other chemicals was confirmed by running reagent blanks before analysis.

2.6 Standard solution

Standard stock solution of (500 ppm) of carbon disulfide was prepared in methanol and serially diluted (0.5 ppm, 0.25 ppm, 0.1 ppm, 0.05 ppm, 0.025 ppm, 0.01 ppm) with methanol from the intermediate stock standard solution (20 ppm) for instrument analysis.

2.7 Extraction and clean up procedure for green chilli, red chilli and soil

For drawing representative samples, green and red chilli fruits were cut into pieces followed by mixing. A representative sample of 5 g of different matrices was taken in 50 mL capacity centrifuge tubes. To this 10 mL of cold water and 20 mL of Iso-octane was added. Centrifuge tubes were shaken vigorously. To this 2 mL 40% $SnCl_2$ (Stannous Chloride) in concentrated HCl was added followed by addition of 2 mL of hydrochloric acid. Close the tubes with lid and shaken properly avoiding any spillage. Tubes were sealed properly with many layers of Teflon tape and incubated at 80°C in water bath for 1 hr. Tubes were taken out and allowed to reach room temperature. Tubes were then transferred to -10°C in deep freezer. From this, 1 mL sample was filled quickly in sample vials and injected immediately in GC-MS for determining the CS_2 concentration (Asensio-Ramos *et al.*, 2010; Sumaiyya Mujawar *et al.*, 2014) [2, 13].

Table 2: GC-MS operating parameters for mancozeb analysis

Instrument	Gas Chromatography-Mass Spectrometry (GC-MS: 2010, Single Quadrupole, Shimadzu)		
Column	Column used was GsBP 5 MS, 30 m x 0.25 mm i.d., 0.25 µm film thickness		
Oven Temperature Programming	100 °C 5.0 °C 290 °C → (0.5 min) 1.0 min (5.0 min)		
Injection Temperature	248 °C		
Gas Flow Rate	Helium	:	1.5 mL min^{-1}
	Total flow	:	17.8 mL min^{-1}
	Column flow	:	0.8 mL min^{-1}
	Mode	:	Split 1:5
GCMS-QP 2010 Parameters	Ion Source Temperature	:	230 °C
	Interface Temperature	:	290 °C
	Solvent Cut Time	:	5.0 min
	Start time (min)	:	0.8
	End time (min)	:	10.5
	Acq. Mode	:	Scan
	Event Time (Sec)	:	0.6
	Scan speed	:	769
	Start m/z	:	50
	End m/z	:	450

2.8 Calculation

$$\text{Residues in ppm} = \frac{H_1 \times V_1 \times C}{H_2 \times W}$$

Where

H_1 = Height of sample
H_2 = Height of standard
V_1 = Total volume of sample in mL
C = Concentration of analytical standard in ppm (µg mL^{-1})
W = Weight of the sample in g

2.9 Linearity

Calibration curve was established with concentrations of the standard and corresponding peak area. The regression coefficient (R^2) obtained from the curve was greater than 0.99 over the range tested. The result of the linearity study proved the methods ability to obtain test results, which are directly proportional to the concentration of analyte in the sample.

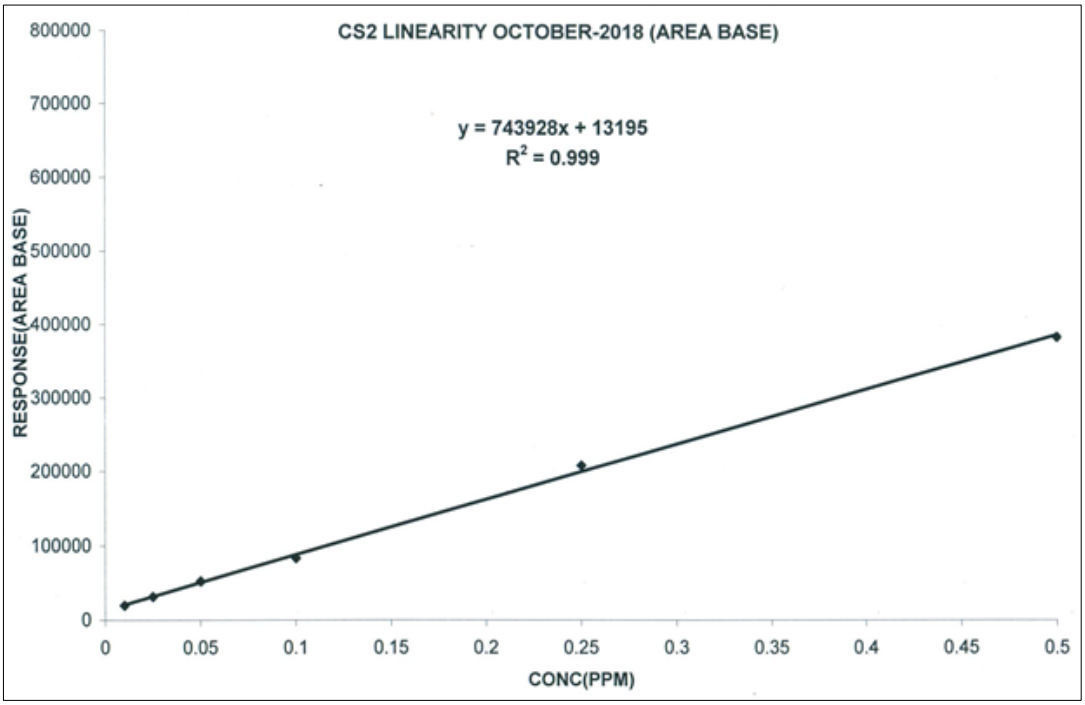

Fig 1: CS2 linearity October-2018 (area base)

2.10 Recovery study

The result revealed that recovery of mancozeb in green chilli, red chilli and soil was within acceptable range of 80 to 120 percent as per SANTE, 2017. Recovery studies were carried out in order to establish the reliability of the method of analysis by fortifying samples collected from untreated control plot. The samples of green chilli, red chilli and cropped soil were spiked by mancozeb standard at three different concentrations *i.e.* 0.05 mg/kg (LOQ Level), 0.25 mg/kg (5 times LOQ level) and 0.50 mg/kg (10 times LOQ level). Results of recovery clearly indicated the fitness of the analytical method for extracting the analyte of the interest from given sample matrix with recovery between 80-120%. The calibration solutions were stored at 4 °C.

Table 3: Per cent recovery of mancozeb in different matrices of chilli and cropped soil

Substrate	Percent Recovery (Mean* ± SD)		
	Fortification Level		
	0.05 mg/kg	0.25 mg/kg	0.50 mg/kg
Green chilli	99.24 (± 3.84)	99.76 (±6.08)	117.65 (±6.06)
Red chilli	89.04 (±5.15)	84.9 (±3.27)	107.52 (±1.80)
Soil	109.27 (±1.69)	93.95 (±6.28)	112.45 (±8.05)

* mean of three replications.

2.10 Statistical analysis

Persistence data were fitted into first order dissipation kinetics. It was subjected to regression analysis by Hoskin (1961) [6] for computing residual half-lives (Gunther and Blinn, 1955) [5].

3. Results and Discussion

3.1 Residues of mancozeb in green chilli, red chilli and soil

The dissipation of green chilli followed first order kinetics wherein mean initial residues of mancozeb (based on CS_2) in green chilli were found to be 0.36 mg/kg at recommended dose and 1.24 mg/kg at double the recommended dose (Table 3 and Fig. 2 & 3) which dissipated to below limit of quantification (0.05 mg/kg) by 15th day with a half-life of 1.326 and 2.076 days at recommended and double the recommended doses, respectively. The residues of mancozeb (as CS_2) in red chilli and soil collected at harvest were found below limit of quantification of 0.05 mg/kg.

Present findings are in agreement with Sarkar *et al.* (2005) [14] who reported that mancozeb residues were found to be below detectable limits on the 5th day for the recommended dose and 7th day for double the recommended dose in the case of onion whole plant with varied half life from 0.97 to 1.22 days. Jagdish *et al.* (2015) [7] reported the initial residue deposits of mancozeb (as CS_2) in tomato crop in two different seasons in the range of 3.41 to 6.07 mg/kg which dissipated after 3 days of application in *kharif* (1.86 mg/kg) and *rabi* (1.79 mg/kg) seasons for the recommended dose. At the harvest time (10 days after the last spray) the residue level reached below

detectable limit of recommended dose in *kharif* (0.1 mg/kg) and *rabi* (0.12 mg/kg), at double the recommended dose the residues were 0.24 and 0.32 mg/kg.

Similarly, according to El Habib Ait Addi, (2017), degradation rate of mancozeb in tomato at two doses under both open field and greenhouse condition followed first order kinetics with halflives of 1.77 and 1.3 days and 2.0 and 1.8 days, respectively.

Devi *et al.* (2015) [3] reported average initial deposition of mancozeb in mango in the range of 2.25 to 2.71 and 4.17 to 5.96 mg/kg at respective doses which dissipated to below detectable limit of 7 days after spray at recommended dosage in all the locations. The fungicide degradation followed a first order kinetics with half-lives of 1-3 days, for mancozeb. Ritu Rani *et al.* (2013) [10] estimated the dissipation of mancozeb and metalaxyl in tomato by following four applications of a combination formulation Ridomil MZ (mancozeb 64% + metalaxyl 8%) at 0.25% and 0.50% at 10 days interval. They further reported half-life periods for mancozeb as 3.7 6 and 4.1 4 days, at single and double the application rates, respectively. Residues of mancozeb dissipated below limit of quantification (LOQ) of 0.25 mg kg^{-1} after 10 and 15 days at single and double the application dosages, respectively. All these reports lend support to the present findings.

Table 4: Dissipation kinetics of mancozeb in green chilli

Days after final spray (56 DAT)	Residues of Mancozeb (mg/kg)									
	X Dose (1500 g *a.i.*/ha)					2X Dose (3000 g *a.i.*/ha)				
	R_1	R_2	R_3	Mean	SD (±)	R_1	R_2	R_3	Mean	SD (±)
0	0.29	0.39	0.40	0.36	0.064	1.42	1.14	1.18	1.24	0.151
1	0.39	0.43	0.48	0.43	0.042	1.04	1.09	1.65	1.26	0.339
3	0.10	0.11	0.13	0.11	0.001	0.38	0.36	0.36	0.36	0.014
5	0.08	0.08	0.08	0.08	0.003	0.29	0.36	0.35	0.33	0.040
7	0.08	0.09	0.08	0.08	0.004	0.10	0.13	0.11	0.11	0.015
10	0.05	0.04	0.04	0.05	0.007	0.05	0.05	0.05	0.05	0.002
15	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Red chilli at harvest (85 DAT)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Soil at harvest (85 DAT)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Regression equation	Y= -0.227x + 2.825					Y= -0.145x + 3.133				
R^2	0.807					0.966				
DT 50	1.326					2.076				

LOQ = Limit of Quantification (<0.05 mg/kg)
DAT = Days after Transplanting

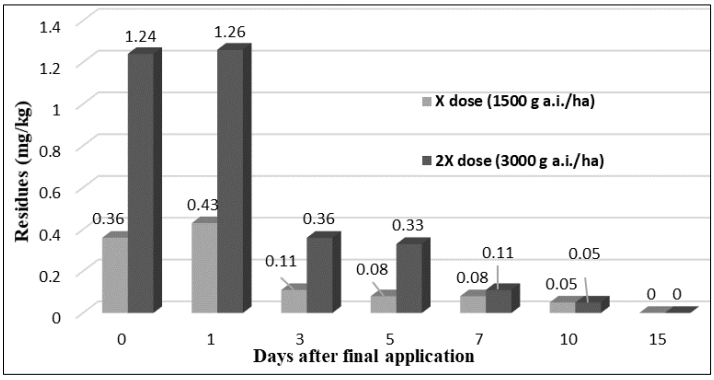

Fig 2: Dissipation kinetics of mancozeb in green chilli

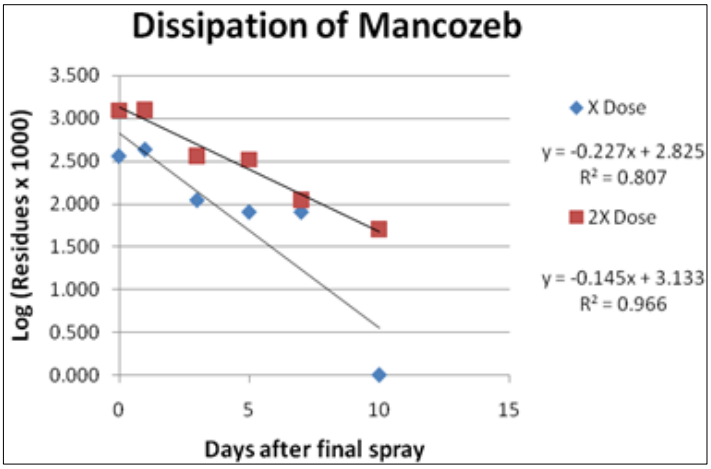

Fig 3: Semi logarithmic graph depicting dissipation kinetics of mancozeb on green chilli

4. Conclusion
The study cleared showed that mancozeb 75% WP has no detectable residues either in green chilli (at 15 days after final spray), red chilli and soil at harvest. It was concluded that mancozeb does not pose any residual toxicity problem in chilli production ecosystem and is safely recommended for use.

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