

Persistence Behaviour of Fungicide Mixture (Benalaxyl-M 4% + Mancozeb 65%) WP in Grapes

Chiranjit Kundu · Arnab Goon · Anjan Bhattacharyya

Received: 24 April 2012 / Accepted: 24 September 2012 / Published online: 2 October 2012
© Springer Science+Business Media New York 2012

Abstract Benalaxyl-M [methyl N-phenylacetyl-N-2, 6-xylyl-DL-alaninate] is a newly introduced phenyl-amide fungicide whereas Mancozeb is one of the member of ethylenebisdiethiocarbamate (EBDC) fungicide. Benalaxyl-M in combination with Mancozeb effectively controls downy mildew of grapes. A two season field trial was conducted under West Bengal climatic condition during 2009–2010 to evaluate the dissipation pattern of fungicide mixture (Benalaxyl-M 4 % + Mancozeb 65 %) WP in grapes at two application rates (2,750 g and 5,500 g ha⁻¹). The quantitative analysis was performed using liquid chromatography–mass spectrometry (LC–MS/MS) and UV–Vis spectrophotometer for Benalaxyl-M and Mancozeb residues respectively. Following the first order kinetics the fungicide Benalaxyl-M dissipates in grapes with half-life ($T_{1/2}$) value ranges between 2.59 and 2.79 days irrespective of seasons and doses. The dissipation pattern of Mancozeb also follows first order kinetics with half-life ($T_{1/2}$) value ranges between 3.86 and 4.93 days irrespective of seasons and doses.

Keywords Persistence · Dissipation · Susceptible · Benalaxyl-M

Grapes (*Vitis vinifera* L.) belong to the world's largest fruit crops with a global production of around 69 million tons in 2006 (FAOSTAT 2007), contain large amounts of phytochemicals including anthocyanins and resveratrol, which

offer health benefits (Pezzuto 2008), suffers yield losses due to fungal diseases. Downy mildew of grapes is caused by fungus *Plasmopara viticola* is a serious problem in most of the grape growing areas of west Bengal.

The efficacy of common fungicides to control downy mildew in vines decreases when the same commercial formulations are applied over several years. For this reason, new commercial formulations (containing new fungicides) have to offer to grape-growers to increase the efficacy of antifungal treatments (Egger 2001).

Benalaxyl-M, discovered by Isagro, is a biological active isomer of Benalaxyl that shares the typical properties of phenylamide fungicides. It is characterized by a favourable pharmacokinetics in plants and an increased activity. Thus, it cuts the application rate of racemic Benalaxyl in half, improving the antiperonosporic performance. The quantity of fungicide applied, amount of residues in crops, and impact on the environment were reduced.

Benalaxyl-M in combination with Mancozeb effectively controls downy mildew of grapes (Gonzalez et al. 2009). The objective of the present work was to study the dissipation and the fate of fungicide mixture Benalaxyl-M + Mancozeb residue in grapes grown in different seasons under West Bengal climatic condition (Table 1).

Materials and Methods

A two season field study was conducted at Malancha Farm, Vill.- Siyan, Dist.- Birbhum during March 2009 to June 2009 (1st season) and March 2010 to June 2010 (2nd season) on grapes [variety- Carolina Black Rose]. The formulation (Benalaxyl-M 4 % + Mancozeb 65 %) WP was applied with the help of knapsack sprayer equipped

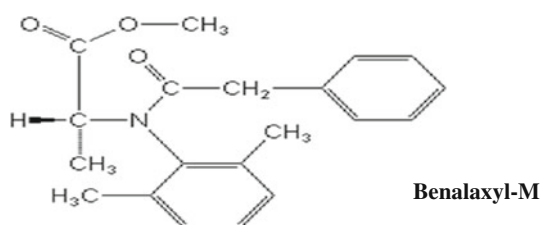
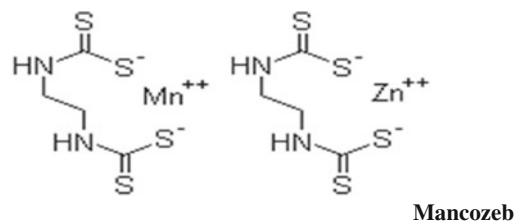
C. Kundu · A. Goon · A. Bhattacharyya (✉)
Pesticide Residue Laboratory, Department of Agricultural
Chemicals, Bidhan Chandra Krishi Viswavidyalaya,
Mohanpur Nadia 741252, West Bengal, India
e-mail: anjan_84@rediffmail.com

with WFN 62 nozzle at $2,750 \text{ g ha}^{-1}$ (T_1) and at $5,500 \text{ g ha}^{-1}$ (T_2) in Randomized Block Designed (RBD) plots along with untreated control (T_3) plots. Spraying of fungicide mixture was done four times at 7 days interval for both the seasons. For persistence study, grapes sample was collected at 0, 1, 3, 5, 10, 15 days after 4th application

of the fungicide mixture for both seasons. Grapes and cropped soil samples (For both seasons) were also collected at the time of harvest following standard sampling procedures. Samples from untreated control plots were also collected in the same way. Samples (plant and soil samples) were air dried to facilitate extraction. Two valid

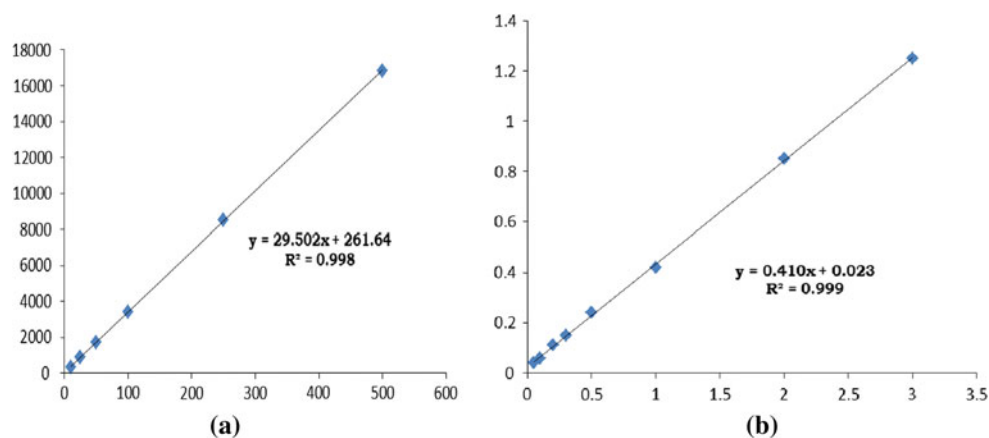
Table 1 Physico-chemical properties of Benalaxyl-M and Mancozeb

1.	IUPAC name	<i>Benalaxyl-M</i> : Methyl N-phenylacetyl-N-2, 6-xylyl-DL-alaninate <i>Mancozeb</i> : Manganese ethylene-bis-(dithiocarbamate) (polymeric) complex with Zn-salt
2.	Empirical formula	<i>Benalaxyl-M</i> : $\text{C}_{20}\text{H}_{23}\text{NO}_3$ <i>Mancozeb</i> : $(\text{C}_4\text{H}_6\text{N}_2\text{S}_4\text{Zn})_x (\text{C}_4\text{H}_6\text{N}_2\text{S}_4\text{Mn})_x$
3.	Molecular weight	<i>Benalaxyl-M</i> : 325.4 <i>Mancozeb</i> : 275.72/265.28
4.	Melting point	<i>Benalaxyl-M</i> : 76.8°C <i>Mancozeb</i> : $192\text{--}194^\circ\text{C}$
5.	Water solubility (at $20^\circ\text{C} + 1^\circ\text{C}$)	<i>Benalaxyl-M</i> : 0.0286 g/L <i>Mancozeb</i> : 0.006 g/L
6.	Vapor pressure	<i>Benalaxyl-M</i> : $5.72 \times 10^{-4} \text{ Pa}$ at 20°C <i>Mancozeb</i> : Negligible at 20°C
7.	Chemical structure	



Source The pesticide manual

Fig. 1 Calibration curve of **a** Benalaxyl-M and **b** Standard solutions of CS_2



representatives (10 g) of both substrates were prepared by quartering technique in the laboratory and taken for final analysis of both the fungicides separately.

For Benalaxyl-M residue analysis 5 g of the homogenized sample (grapes and soil sample separately) 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 adding 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 and 25 mg PSA was added to it and vortex for 2 min and the sample was again centrifuged for 10 min at 5,000 rpm. Then 3 mL supernatant liquid was collected from it and evaporated to dryness in N_2 -evaporator at 25°C . The residue was then reconstituted in 3 mL of $[\text{MeOH}: \text{H}_2\text{O} (9:1, \text{v/v}) + 5 \text{ mM } \text{CH}_3\text{COONH}_4]$. The sample was then filtered through 0.2μ membrane filter. The quantitative analysis of Benalaxyl-M was performed by liquid chromatography–tandem mass spectrometry. The HPLC separation was performed by injecting 20 μL via autosampler on a Symmetry C_{18} (5 μm ; $2.1 \times 100 \text{ mm}$) column (Waters, USA) at the flow rate of 0.2 mL/min. The mobile phase was composed of (A) methanol/water 10/90 (v/v) with 5 mM ammonium acetate and (B) methanol/water 90/10 (v/v) with 5 mM ammonium acetate. The optimized MS instrument parameters includes: capillary voltage, 1.20 kV; cone voltage, 18 V; source temperature, 120°C ; desolvation temperature, 350°C ; desolvation gas flow, 650 L/h nitrogen; cone gas flow, 25 L/h argon; collision gas pressure to 3.5×10^{-3} psi for MS/MS. Quantification was performed by multiple reaction monitoring (MRM) with two mass transition ($325.4 \rightarrow 208.0$ and $325.4 \rightarrow 148.0$). The limit of detection (LOD) and limit of quantification (LOQ) for Benalaxyl-M were found 0.005 and 0.01 $\mu\text{g/g}$, respectively.

For the analysis of Mancozeb residue 5 g of homogenized sample (grapes and soil sample) was taken separately (replication and treatment wise) in a three necked round bottom flask. 200 mL distilled water, 20 mL conc. HCl and 20 mL SnCl_2 (40 %) were added to the flask and swirled for 2 min for each soil and grapes sample. The mixture was then digested to evolve CS_2 . A water condenser was connected to the middle neck and the outlet was connected to the two tubes containing NaOH and Lead Acetate solution (30 %) in series for cleanup of the CS_2 evolved which forms a complex with the coloring agent kept in the third tube connected to the Lead acetate tube. The mixture was then diluted with ethanol and was analyzed in the UV–VIS spectrophotometer at λ_{max} 435 nm. The quantitative analysis of Mancozeb was performed with Varian CARY 50 UV–VIS spectrophotometer which is controlled by Cary WinUV software at the maximum absorption band at 435 nm. The limit of detection (LOD) and limit of

Table 2 Recovery study of Benalaxyl-M in different substrates of grapes

Substrate	Amount fortified ($\mu\text{g/g}$)	Amount recovered ^a ($\mu\text{g/g}$)	% Recovery	Average % recovery
Grapes sample	0.01	0.009	90.00	89.04
	0.10	0.086	86.00	
	0.50	0.456	91.13	
Field soil	0.01	0.009	90.00	91.87
	0.10	0.089	89.00	
	0.50	0.483	96.60	

^a Average of three replicates

Table 3 Recovery study of Mancozeb in different substrates of grapes

Substrate	Amount fortified ($\mu\text{g/g}$)	Amount recovered ^a ($\mu\text{g/g}$)	% Recovery	Average % recovery
Grapes sample	0.10	0.090	90.00	94.60
	0.50	0.483	96.60	
	1.00	0.972	97.20	
Field soil	0.10	0.094	94.00	95.57
	0.50	0.484	96.80	
	1.00	0.959	95.90	

^a Average of three replicates

quantification (LOQ) for Mancozeb were found 0.05 and 0.1 $\mu\text{g/g}$, respectively.

The calibration curve was constructed by plotting relevant standard concentration versus absorption for both Benalaxyl-M and Mancozeb separately (Fig. 1).

Recovery studies were carried out for both Benalaxyl-M and Mancozeb separately 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 field soil and plant (Grapes) samples with different levels of both the analytical standard solution separately. Results of recovery study are shown in Tables 2 and 3.

Results and Discussion

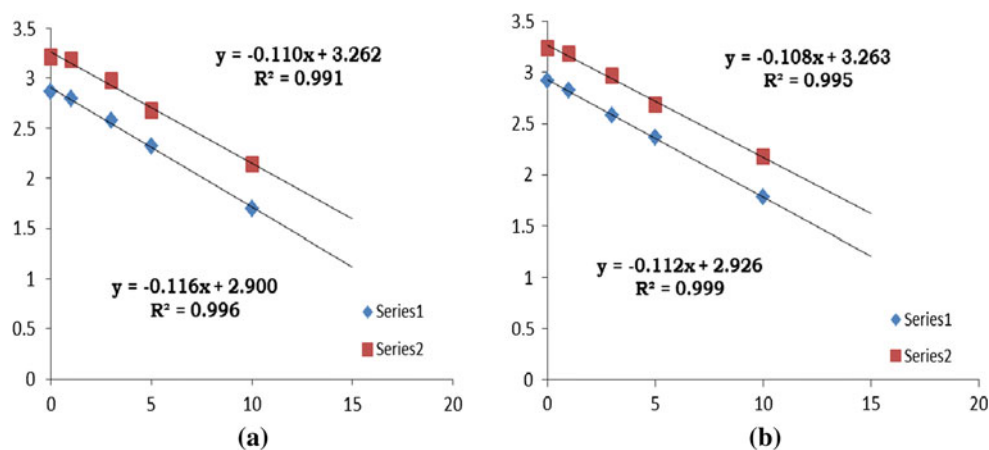
The recovery percentage of Benalaxyl-M in grapes and field soil were 89.04 % and 91.87 % respectively (Table 2). Similarly the recovery percentage of Mancozeb in grapes and field soil were 94.60 % and 95.57 % respectively (Table 3). Hence the methods can be adopted.

The results of field study of persistence of Benalaxyl-M in grapes for both seasons have been summarized in

Table 4 Dissipation of Benalaxyl-M in Grapes Sample for both seasons

Days after application	Treatment	Residues in µg/g (Season-I)		Residues in µg/g (Season-II)	
		Mean ± SD	Dissipation (%)	Mean ± SD	Dissipation (%)
0	T ₁	0.74 ± 0.029	–	0.83 ± 0.037	–
1		0.63 ± 0.022	14.86	0.67 ± 0.029	19.28
3		0.38 ± 0.016	48.65	0.38 ± 0.033	54.22
5		0.21 ± 0.025	71.62	0.23 ± 0.032	72.29
10		0.05 ± 0.005	93.24	0.06 ± 0.005	92.77
15		BDL	–	BDL	–
0	T ₂	1.64 ± 0.016	–	1.74 ± 0.046	–
1		1.54 ± 0.041	6.09	1.51 ± 0.053	13.22
3		0.95 ± 0.012	42.07	0.93 ± 0.042	46.55
5		0.48 ± 0.013	70.73	0.48 ± 0.037	72.41
10		0.14 ± 0.021	91.46	0.15 ± 0.021	91.38
15		BDL	–	BDL	–

BDL below detectable limit

Fig. 2 Linear plot of dissipation of Benalaxyl-M in grapes in **a** season-I and **b** season-II**Table 5** Dissipation of Mancozeb in Grapes Sample for both seasons

Days after application	Treatment	Residues in µg/g (Season-I)		Residues in µg/g (Season-II)	
		Mean ± SD	Dissipation (%)	Mean ± SD	Dissipation (%)
0	T ₁	0.30 ± 0.017	–	0.32 ± 0.017	–
1		0.23 ± 0.016	23.33	0.25 ± 0.016	21.88
3		0.18 ± 0.012	40.00	0.19 ± 0.016	40.63
5		0.12 ± 0.008	60.00	0.12 ± 0.005	62.50
10		BDL	–	BDL	–
15		BDL	–	BDL	–
0	T ₂	0.66 ± 0.025	–	0.65 ± 0.028	–
1		0.51 ± 0.021	22.72	0.51 ± 0.016	21.54
3		0.38 ± 0.025	42.42	0.37 ± 0.012	43.08
5		0.26 ± 0.024	60.60	0.27 ± 0.017	58.46
10		0.15 ± 0.016	77.27	0.15 ± 0.012	76.92
15		BDL	–	BDL	–

BDL below detectable limit

Table 4. It was found that the residues gradually decreased with time following 1st order kinetics. The initial deposits (2 h after spraying) of Benalaxyl-M in grapes for 1st season were found 0.74 µg/g (T₁) and 1.64 µg/g (T₂) and the

half-life values (T_{1/2}) were found to be 2.59 days (T₁) and 2.73 days (T₂). In case of 2nd season the initial deposits (2 h after spraying) of Benalaxyl-M in grapes were found 0.83 µg/g (T₁) and 1.74 µg/g (T₂) and the half-life values

Fig. 3 Linear plot of dissipation of Mancozeb in grapes in **a** season-I and **b** season-II

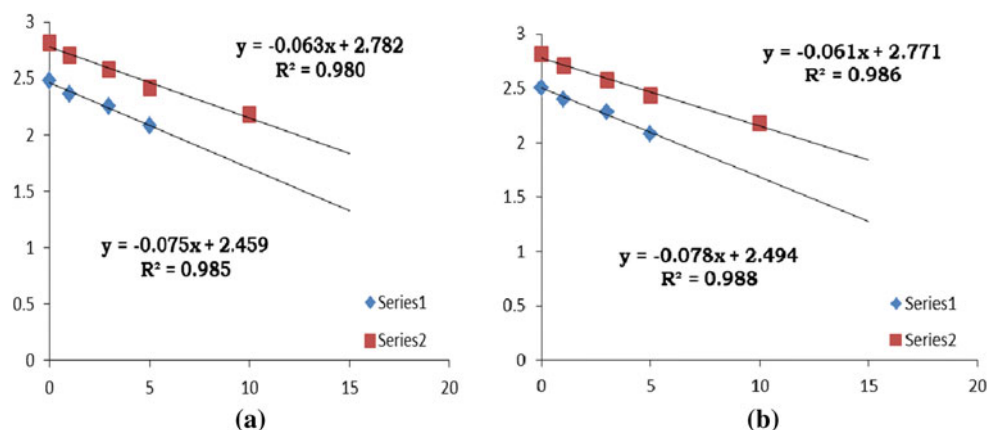


Table 6 Results of statistical interpretation of residue data

Substrate	Regression equation	Half-life ($T_{1/2}$)[days]
Benalaxyl-M(season-I)	$y = 2.900 - 0.116x$ (T_1)	2.59–2.73
	$y = 3.262 - 0.110x$ (T_2)	
Benalaxyl-M(season-II)	$y = 2.926 - 0.112x$ (T_1)	2.68–2.79
	$y = 3.263 - 0.108x$ (T_2)	
Mancozeb(season-I)	$y = 2.459 - 0.075x$ (T_1)	4.01–4.78
	$y = 2.782 - 0.063x$ (T_2)	
Mancozeb(season-II)	$y = 2.494 - 0.078x$ (T_1)	3.86–4.93
	$y = 2.771 - 0.061x$ (T_2)	

($T_{1/2}$) were found to be 2.68 days (T_1) and 2.79 days (T_2). More than 50 % of the initial deposit was dissipated within 5 days irrespective of any doses and seasons. The dissipation patterns as well as half-life values of the present study are in well agreement with the earlier studies conducted in grapes (Cabras et al. 2010). Monchiero et al. (1996) reported the same trend in their studies of results of control trials of Benalaxyl against *Plasmopara viticola* in Piedmont (Fig. 2).

The results of field study of persistence of Mancozeb in grapes for both seasons have been summarized in Table 5. The initial deposits (2 h after spraying) of Mancozeb in grapes for 1st season were found 0.30 $\mu\text{g/g}$ (T_1) and 0.66 $\mu\text{g/g}$ (T_2) and the half-life values ($T_{1/2}$) were found to be 4.01 days (T_1) and 4.78 days (T_2). In case of 2nd season the initial deposits (2 h after spraying) of Mancozeb in grapes were found 0.32 $\mu\text{g/g}$ (T_1) and 0.65 $\mu\text{g/g}$ (T_2) and the half-life values ($T_{1/2}$) were found to be 3.86 days (T_1) and 4.93 days (T_2). More than 50 % of the initial deposit was dissipated within 5 days irrespective of any doses and seasons. The dissipation patterns as well as half-life values of the present study are in well agreement with the earlier studies conducted in grapes under semi-arid tropical climatic condition (Navarro et al. 2001). Banerjee et al. (2008) stated the same trend in their studies in Persistence and residue

dynamics of Mancozeb and its toxic metabolite ethylene thiourea (ETU) in table grapes. Dethe et al. (1993)??? also reported the same trend in their studies of residues of fungicides used against downy mildew of grapes (Fig. 3).

No residues of both the fungicide were detected in harvest grapes and soil samples irrespective of any doses and seasons. Similar observations were also reported earlier by Gozzo et al. (1988) as they described the chemical evidence of the effects of Mancozeb on Benalaxyl in grape plants as possible rationale for their synergistic interaction (Table 6).

References

- Banerjee K, Oulkar DP, Adsule PG (2008) Persistence and residue dynamics of Mancozeb and its toxic metabolite ethylene thiourea (ETU) in table grapes. *Acta Hort* 785:409–412
- Cabras P, Angioni A (2000) Pesticide residues in grapes, wine, and their processing products. *J Agric Food Chem* 48(4):967–973
- Dethe MD, Rane SD, Kale VD (1993) Residues of fungicides used against downy mildew of grapes. *J Maharashtra Agric Univ* 18(1):150–151
- Egger E (2001) Trends and new products for the protection of grapevines. *Infor Agrar* 57(15):75–86
- FAOSTAT (2007) FAO Statistical Database. <http://www.fao.org>. Accessed on 4 May 2009
- Gonzalez Rodriguez RM, Cancho Grande B, Simal Gandara J (2009) Efficacy of new commercial formulations to control downy mildew and dissipation of their active fungicides in wine after good agricultural practices. *J Sci Food Agric* 89(15):2625–2635
- Gozzo F, Pizzingrilli G, Valcamonica C (1988) Chemical evidence of the effects of mancozeb on benalaxyl in grape plants as possible rationale for their synergistic interaction. *Pesticide Biochem Physio* 30(2):136–141
- Monchiero M, Gullino ML, Garibaldi A (1996) Results of control trials against *Plasmopara viticola* in Piedmont. *Infor Fitopatologico* 46(11):46–48
- Navarro S, Oliva J, Navarro G, Barba A (2001) Dissipation of chlorpyrifos, fenarimol, mancozeb, metalaxyl, penconazole, and vinclozolin in grapes. *Am J Enol Vitic* 52(1):35–40
- Pezzuto JM (2008) Grapes and human health: a perspective. *J Agric Food Chem* 56:6777–6784
- Tomlin CDS (1997) The pesticide manual. British Crop Product Council, Surrey