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Field Bio-efficacy and Residue Dynamics of the Fungicide Polyoxin D Zinc Salt 5% SC in Grape (*Vitis vinifera*)

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Abstract

The field experiments were carried out to evaluate the bio-efficacy and residue dynamics of Polyoxin D Zinc salt 5% SC in grape during 2014–2015 and 2015–2016 at ICAR-National Research Centre for Grapes, Pune. Polyoxin D Zinc salt 5% SC @ 600 ml ha^{-1} gave the best control of the disease, both in the leaves and bunches with a percent disease control of 56.4 and 75.7 respectively, as compared to untreated control. The percent disease control of the test fungicide Polyoxin D Zinc salt 5% SC @ 600 ml ha^{-1} was superior to all the triazoles viz. Flusilazole 40 EC, Hexaconazole 5 EC and Myclobutanil 10 WP, used in the study. The yield data reflected a similar trend wherein the maximum percent increase in yield was observed in case of Polyoxin D Zinc salt 5% SC @ 600 ml ha^{-1} i.e. 57.47 as compared to untreated control. However, all the triazoles manifested a higher percent increase in yield as compared to the lowest dose of the test fungicide i.e. 200 ml ha^{-1}. For the detection and quantification of polyoxin D residue in grape, we have developed an efficient and effective analytical method, using liquid chromatography-tandem mass spectrometry (LC-MS/MS), in field treated samples. The residue data had excellent fit to 1^{st}+1^{st} order models giving r^2 value of >0.99 with a half-life ($t_{1/2}$) 8.0 days for recommended dose and 14.5 days for double dose. These findings are useful for effective disease management in grape crop amalgamated with food safety and consumer satisfaction.

Keywords: Bioefficacy, grape, polyoxin D, powdery mildew, residues

1. Introduction

Grape (*Vitis vinifera*; Family: Vitaceae) is a fruit crop of commercial significance in the arena of Indian horticulture garnering an export value of 294.63 million USD (Anonymous, 2019) for the national exchequer. It is consumed as fresh fruits as well as in the processed forms like juice, wine, raisins etc. One of the major bottlenecks of grape production is powdery mildew disease caused by the ascomycetous pathogen *Erysiphe necator* Schwein. [Previously known as *Uncinula necator* (Schwein.) Burril] which is capable of infecting the crop from flowering to fruit setting in most of the grape-growing areas of the world, including tropics (Reddy et al., 2017). Failure or partial control of the disease results in plummeting of yield and quality (Calonnec et al., 2004) of the produce. Fungicides are the major arsenal to combat the menace of powdery mildew. Sulphur (Pearson and Goheen, 1988; Keller and Hrazdina., 1988), Sterol biosynthesis inhibitors (Chao et al., 2011; Dutzmann et al., 1996), strobilurins

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(Bartlett et al., 2002) and succinate dehydrogenase inhibitors (Sierotzki and Scalliet, 2013; Stammler et al., 2015) have been used successfully in controlling the disease but repeated applications of chemical fungicides result in development of pathogen resistance (Ghule et al., 2018) and festered environmental conditions (Thomas, 1986). Hence, a tectonic shift towards the use of effective but safe chemicals is the need of the hour and polyoxins produced by *Streptomyces cacaoi* var. *asoensis* (Isono et al., 1965) could be such a solution for the management of powdery mildew in grapes.

Polyoxins, are novel antifungal antibiotics containing at least 12 active (Pol-A to Pol-P) components. Among them, Polyoxin D, (also known as polyoxorim) is responsible for inhibiting chitin synthetase thereby impairing the biosynthesis of chitin in the fungal cell wall (Endo et al., 1970). This inhibition was possible by deterring the incorporation of 14C glucosamine into the fungal cell wall (Cabib, 1991). Polyoxin D (zinc 5 - [[2-amino-5-O (aminocarbonyl)-2-deoxy-Lxylonoyl] amino]-1-(5-carboxy-3,4-dihydro-2,4-dioxo-1(2H)-pyrimidinyl) -1, 5dideoxy-β-D-allofuranuronate) is highly water soluble and so its zinc salt formation is used to give longer residence time on plant surfaces (Anonymous, 2012). It has a proven record of being highly effective in controlling fungal diseases over a vertical of crop plants like rapeseed, peach, melons and paddy (Isono and Suzuki, 1968; Tewari and Skoropad, 1979; Hao et al., 2017; Keinath, 2016 Yamaguchi, 1998; Adaskaveg et al., 2014; Adaskaveg and Forster, 2014). Polyoxin D zinc salt with a unique, non-toxic mode of action [Fungicide Resistance Action Committee (FRAC) Code 19] is claimed to be effective against powdery mildew of grapes but data under tropical conditions are wanting. However, recently its compatibility was tested with major biocontrol agents used in viticulture and it was reported to be compatible with most potential components in grape IPM viz, *Trichoderma viride, Bacillus subtilis, Pseudomonas fluroscence, Ampelomyces quisqualis, Metarhizium anisopliae* and *Paecilomyces lilacinus* (Saha et al., 2020).

Fungicide residues and their persistence on or in food are important and well-known concern for human health and environmental safety. The fungicide dissipation processes are complex and depend not only on their physicochemical properties, but also in their degradability by biotic and abiotic processes (Navarro et al., 2001; Farha et al., 2016). Once their intended action is accomplished, they should dissipate. It is generally assumed that the kinetics of these processes follow a pseudo 1st-order equation that depends solely on the concentration of the compound under study. This assumption allows the determination of the half-life (time needed to reduce the concentration of the pesticide in the matrix under study to 50% of its original concentration), an important parameter that is used to establish the pre-harvest interval as well as the adjustment to accomplish the legal MRLs (Maximum Residue Limit) to ensure safe consumption (Karmakar and Kulshrestha, 2009).

To the best of our knowledge, studies on the dissipation behavior of polyoxin D on grapes under field conditions have not been reported in the literature. It was extremely challenging to recover polyoxin D due to its hydrophilic nature. However, polyoxin D is highly affected by the matrix interference in terms of signal suppression which led to ambiguous detection. So, here we propose an optimized and validated analytical method for the detection and quantification of polyoxin D in grape using LC-MS/MS (Liquid Chromatography with Tandem Mass Spectrometry) to determine its half-life and dissipation pattern.

This antibiotic is yet to have a label claim on grape so evaluating the bioefficacy at the optimum dose is a prerequisite of the regulatory process. This study was aimed to fortified with residue dissipation patterns, half-life, pre-harvest interval (PHI) and risk assessment of the fungicide on the harvestable produce so as to ensure its successful incorporation in the good agricultural practices of the grapes.

2. Materials and Methods

The experiment was conducted in vineyards of Tas-A-Ganesh variety grown on Bower system of training at ICAR-National Research Centre for Grapes, Pune (latitude: 18.52°N, longitude: 73.86°E, elevation: 560 m MSL) India during two seasons (2014–15 and 2015–16).

2.1. Chemicals and reagents

The commercial formulation of Polyoxin D zinc salt 5% SC was received from PI Industries Limited, Udaipur, India for evaluation against Powdery mildew in grape. All the reagents and solvents used in residue analysis were of AR and gradient grade and procured from Merck India Ltd. (Mumbai). A Sartorius (Gottingen, Germany) water purification system was used to generate HPLC grade water. The apparatus used for sample preparation included a mixer with grinder (0.5 and 2 L capacity, model GX7, Bajaj India Ltd., Mumbai), vortex mixer (Genie 2T, Imperial Biomedicals, Mumbai, India), ultrasonic bath (Oscar Electronics, Mumbai, India), low volume concentrator (TurboVap LV; Caliper Life Sciences, Russelsheim, Germany), high speed refrigerated centrifuge (Kubota 6500, Kubota Corp., Tokyo, Japan), and a microcentrifuge (Microfuge Pico, Kendro D-37520, Osterode, Germany). Ultipor Nylon-6,6 membrane filters (0.2 mm pore size and 13 mm diameter) were purchased from Agilent Technologies (Santa Clara, CA, USA) and Pall Life Sciences (Ann Arbor, MI, USA), respectively.

2.2. Standard stock solutions

The Polyoxin D zinc salt 5%SC standard stock solution was prepared by accurately weighing 10 (±0.1) mg in a volumetric flask (certified A class) and dissolving in 10 ml of deionised water. The stock solution was then stored at -20°C. The calibration standards ranging between 1.0 and 50.0 ng ml^{-1} were prepared by serial dilution of a working standard mixture (1 mg ml^{-1}) with methanol/ 20 mM ammonium formate in water (1:1 v/v) or control matrix extracts (for matrix matched standard solutions).

2.3. Test fungicides against powdery mildew

Seven treatments comprising of Polyoxin D zinc salt 5% SC at 200, 400 and 600 ml ha^{-1}, Flusilazole 40% EC at 50 ml ha^{-1}, Hexaconazole 5EC at 1000 ml ha^{-1}, Myclobutanil 10% WP at 200 ml ha^{-1} and untreated control were laid down in the randomized block design (RBD) with four replications. Fungicide application was commenced with the visibility of initial symptoms (65 and 68 days after fruit pruning in 2014–15 and 2015–16 respectively) and two curative sprays were given at an interval of 15 days with knapsack sprayer. Water volume used for spray was calculated based on requirement of 1000 l ha^{-1} at full canopy.

Powdery mildew incidence on leaves was recorded adopting 0-4 scale, where 0 means no disease present and 4 means more than 75 per cent leaf area infected (Ghule et al, 2018). Percent Disease Index (PDI) was calculated by following formulae of Wheeler (1969).

PDI=(Sum of numerical ratings×100)/(Number of leaves observed×Maximum rating)

The ratings on minimum ten leaves and a bunch were recorded on randomly selected canes. Ten such canes per plant were observed. The observations on 8 plants spread over four replication were recorded. Thus, the data presented on PDI is average of randomly selected 80 canes. After maturity, the bunches were harvested and fruit yield was calculated in tones per hectare.

The mean of PDI of both the seasons was calculated and percent disease control was tabulated using the formula of Vincent (1947)

I=C–T/C×100

I=percent disease control; C= PDI in untreated control;

T=PDI in fungicide treatment

All data obtained were subsequently analyzed statistically.

2.4. Residue monitoring and sampling

The rates of Polyoxin D zinc salt 5% SC used in the residue dissipation experiments were 30 g a.i.$^{-1}$ (recommended dose) and 60 g a.i. ha^{-1} (double the recommended dose). The berry samples (250 g per replication per treatment) were collected at 0, 1, 3, 5, 7, 10, 15, 21, 30, 45 days and at harvest (60 days after final spraying) as described by Oulkar et al. (2009) randomly from each replicate after application of final spray. Samples from the control plots were simultaneously collected in the same way and all the treatments were replicated thrice. The samples were collected in polythene bags and stored at -20°C until analysis to avoid any degradation.

2.4.1. Sample preparation and extraction

Entire 250 g sample was crushed in a homogenizer thoroughly and 10 g homogenized sample was taken in a centrifuge tube and 20 ml acidified Methanol (0.1% Formic Acid) was added. The mixture was homogenized for 1 min and then centrifuged for 5 minutes at 5000 rpm. One ml clear supernatant was taken in eppendorf tube and again centrifuged for 5 minutes at 10000 rpm. The 500 µl supernatant was taken in fresh eppendorf tube to which 500 µl water was added. Mixture was then sonicated and homogenized. Further, the extract was filtered through 0.2 µm nylon membrane filter and subsequently injected to LC-MS/MS for residue analysis.

2.4.2. LC-MS/MS analysis

An LC-MS/MS system comprising Agilent HPLC (Agilent, USA) hyphenated to API 4000 triple quadrupole mass spectrometer (Sciex, Foster City, CA, USA) was used for quantification of the residues. The chromatographic separation was carried out using a Princeton SPHER-60 C18 (50×2.0 mm^2 i.d., 3 µm). The mobile phase was composed of (A) in water and (B) in methanol, with 5 mM ammonium formate with 0.1% formic acid. The gradient program for mobile phase was 0–0.5 min 90% A phase, 0.5–2 min 90-30% A phase, 2–7 min 30% A phase, 7–7.5min 30–90% A phase and 7.5–10 min 90% A phase. The column oven temperature was maintained at 40°C. The flow rate was maintained at 350 µl min^{-1}. An aliquot of 5 µl was injected through an autosampler. The polyoxin D sample produced intense signals at m/z values of 84, 102, 305, and 461. The ion peaks at m/z 461 and 305 were selected as the quantifying and qualifying ions, respectively (Table 1). The ion ratio of the two mass transitions was considered for unambiguous identification of the pesticide as per the criteria of the guideline document on analytical quality control and validation procedures for pesticide residue analysis in food and feed, i.e. European Commission, 2017.

Table 1: Fragmentation pattern and mass transition parameters of polyoxin D in grape

MW	Precursor ion (m/z)	Product ion (m/z)	DP	EP	CE	CXP
521	522	Quantitation	461	70	10	25	22
		Confirmation	305	70	10	31	36

2.4.3. Method validation

The single laboratory validation approach was implemented for the validation of residue analysis method. The limits of detection (LOD) and quantification (LOQ) were determined by considering signal-to-noise ratios of 3 and 10, respectively, using matrix matched standards, with recoveries of >70%. The matrix effect (ME) was estimated by comparing the detector response of calibration standards at different levels in solvent versus matrix. The matrix matched calibration standards were prepared using a control grape sample which was previously analyzed to confirm the absence of the test compound. Recovery experiments were carried out by fortifying the untreated crushed grape sample in six replicates with polyoxin D at three concentration levels viz. 0.01, 0.02 and 0.05 mg kg^{-1}. The precision in terms of repeatability was determined by calculating RSD (%) values

associated with recovery.

The overall matrix effect (ME%) was evaluated using the following equation:

ME%=(Peak area of matrix-matched standard-Peak area of solvent standard)×100)/(Peak area of matrix-matched standard)

2.4.4. Statistical analysis

The residue data was assessed by linear as well as non-linear kinetics using the following mathematical equation expressions:

First-order model : $[A]_t=[A]_1 \exp(-k_1 t)$
First+First-order model : $[A]_t=[A]_1 \exp(-k_1 t)+[A]_2 \exp(-k_2 t)$

Where,
$[A]_t$=Concentration (mg kg^{-1}) at time t (days),

$[A]_1$, $[A]_2$=(0 day) degraded through first-order processes,

k_1 and k_2=Degradation rate constants .

In first-order model, the half-life (DT_{50}) was determined as $DT_{50}=\ln(2)\times k_1^{-1}$.

PHI=[log (Intercept) −log (MRL)]/slope of a first-order equation

The equation parameters were calculated by using a commercially available programme Table Curve 2D (v 5.01).

3. Results and Discussion

3.1. Field efficacy of the test fungicide against powdery mildew

In leaves, the test fungicide 600 ml ha^{-1}, manifested the lowest PDI values in both 2014–15 and 2015–16 i.e. 8.12 and 10.19 respectively with a mean percent disease control (PDC) of 56.4 (Table 2) It was followed by its lower dose i.e. 400 ml ha^{-1} where the mean PDC was 43.35. Both the doses proved to be superior to the standard check fungicides myclobutanil 10% WP, flusilazole 40% EC and hexaconazole 5% EC which exhibited a PDC of 20.43, 24.44 and 38.44 respectively. However, the lowest dose of polyoxin –D Zinc salt 5% SC i.e. 200 ml ha^{-1} had a PDC of 34.77 which was almost equal to the PDC of Hexaconazole 5% EC. The untreated control had the maximum PDI of 24.1% and 30.2 in the two consecutive seasons under study. The trend was similar in bunches, where polyoxin –D Zinc salt @ 600 ml ha^{-1} had the lowest PDI in both the seasons i.e. 5.97 and 12.91 with a mean PDC of 75.7, as compared to untreated control. It was followed by the lower dose of the test fungicide i.e. 400 ml ha^{-1} with a PDC of 68.64, but the lowest dose of 200 ml ha^{-1} was inferior to Hexaconazole 5% EC and almost same as that of Myclobutanil 10% WP. The untreated control had the highest PDI of both 38.85 in both the seasons.

The reduction in disease incidence by polyoxin –D Zinc salt 5% SC in also reflected in the yield of the crop, where in the dose of 600 ml ha^{-1} and 400 ml ha^{-1} manifested 57.47% and 40.5% increase in yield respectively, as compared to untreated control (Table 3). The standard check fungicides however yielded higher than the lowest dose of polyoxin-D zinc salt. The pooled data of two years showed the similar trend in case of disease control and yield.

Polyoxin D zinc salt 5% SC is reported to be sensitive to powdery mildew as it controlled the rate of germination and germ tube length of *Sphaerotheca macularis* f. sp. *fragariae*

Table 2: Bio-efficacy of Polyoxin –D zinc salt 5% SC against powdery mildew in grapes after fruit pruning

Treatments	Dose (ml ha^{-1})	PDI of powdery mildew on leaves				PDI of powdery mildew on bunches			
		2014–15	2015–16	Mean	PDC	2014–15	2015–16	Mean	PDC
Polyoxin –D zinc salt 5% SC	200	4.83 (12.61)	6.56 (14.78)	5.69 (13.69)	34.77	4.00 (11.53)	9.69 (18.13)	6.84 (14.83)	61.82
Polyoxin –D zinc salt 5% SC	400	3.31 (10.48)	5.31 (13.31)	4.31 (11.89)	43.35	2.75 (9.53)	6.56 (14.83)	4.65 (12.18)	68.64
Polyoxin –D zinc salt 5% SC	600	2.00 (8.12)	3.13 (10.19)	2.56 (9.15)	56.40	1.08 (5.97)	5.31 (12.91)	3.19 (9.44)	75.70
Myclobutanil 10% WP	200	6.17 (14.38)	10.63 (19.02)	8.4 (16.7)	20.43	3.25 (10.34)	11.25 (19.59)	7.25 (14.96)	61.49
Flusilazole 40% EC	50	6.33 (14.57)	8.75 (17.15)	7.54 (15.86)	24.44	4.38 (12.07)	10.31 (18.71)	7.34 (15.39)	60.38
Hexaconazole 5% EC	1000	4.42 (12.13)	5.63 (13.71)	5.02 (12.92)	38.44	3.17 (10.13)	6.88 (15.19)	5.02 (12.66)	67.41
Untreated control	--	16.67 (24.10)	25.31 (30.20)	20.99 (27.15)	00.00	39.37 (38.85)	39.37 (38.85)	39.37 (38.85)	0.00
SEm±		0.52	0.78	0.39	-	0.45	0.29	3.44	
CD (*p*=0.05)		1.55	2.27	1.19	--	1.32	0.89	1.15	--

*Figure in parenthesis shows angular transformed values

Table 3: Effect of Polyoxin –D zinc salt 5% SC on harvestable yield of grapes

Treatments	Dose (ml ha^{-1})	Harvestable yield (t ha^{-1})			
		2014–15	2015–16	Mean	% increase in yield
Polyoxin –D zinc salt 5% SC	200	13.12	11.29	12.20	29.37
Polyoxin –D zinc salt 5% SC	400	14.42	12.09	13.25	40.50
Polyoxin –D zinc salt 5% SC	600	15.59	14.11	14.85	57.47
Myclobutanil 10% WP	200	13.50	11.20	12.35	30.96
Flusilazole 40% EC	50	14.03	11.38	12.70	34.67
Hexaconazole 5% EC	1000	14.06	12.15	13.10	38.91
Untreated control	--	10.42	8.44	9.43	--
SEm±	--	0.5	0.52	0.39	--
CD (*p*=0.05)	--	1.49	1.12	1.19	--

causing powdery mildew of strawberry (Amsalem et al., 2014). It was sensitive to both the populations, temperate and Mediterranean regions where in the mediterrranean isolate was resistant to other chemicals. Polyoxin D is a competitive inhibitor for UDP-GlcNAc in the chitin synthetase reaction as their chemical structure closely resemble each other (Isono and Suzumi, 1968) Endo et al. (1970) reported the formation of protoplast like structure in *Cochliobolus miyabeanus* which suggested that there is an imbalance between growth of the cell wall which was blocked by Polyoxin D and growth of other cellular structures, which seemed to be unaffected by the antibiotic. Polyoxin B 10% and Polyoxin D 2.2% strongly inhibited the germination and viability of *Alternaria brassicae* conidia under *in vitro* conditions as well as exhibited strong therapeutic action against black spot disease of rapeseed caused by the fungus (Tewari and Skoropad, 1979). However, they pointed out, that the activity of the Polyoxin in the plants declines with time, as they are highly water soluble and are leached out by rain. In the present study, the zinc salt is used which has an advantage of having longer residual time on leaf surfaces and repeated application may not be necessary. Polyoxin –D zinc salt was reported to be equal to or superior in efficacy as compared to penthiopyrad, a new succinate dehydrogenase inhibitor (SDHI) fungicide for the control of *Colletotrichum* sp. causing anthracnose of peaches (Hao et al., 2017). Keinath (2016) opined that Polyoxin D could be used to prevent outbreaks of gummy stem blight on water melons or musk melon seedlings, but it was not able to control *Colletotrichum orbiculare* causing anthracnose of cucurbits, under glass house conditions. The plethora of disease controlled by Polyoxin D along with it being considered to have a medium risk of resistance development (Highland et al., 2014) makes it apt to be included in the package of practice of grapes for an effective control of powdery mildew.

3.2. Residue analysis

3.2.1. Optimization of extraction

Firstly, modified QuEChERS (quick, easy, cheap, effective, rugged, and safe) method of extraction was performed. Grape samples were extracted using 0.1% FA in acetonitrile and salts, and were purified by dispersive-solid phase extraction (d-SPE) using primary-secondary amine (PSA). Instead of a clean chromatogram which was expected for polyoxin D, a significant amount of interference was observed at retention time, though it was fortified with matrix. The target analyte recovery was very poor (<20%). This may be due to the hydrophilic characteristics of polyoxin D, resulting in its failure to partition from the aqueous phase to the organic phase.

A switch-over was made to methanolic extraction method (Oulkar and Banerjee, 2011) for polar compounds extraction. The method involved acidified methanol extraction without purification which gave 83.76% recovery with fortified matrix. So, the solvent composition used in the extraction step had a significant influence on the recovery of the analyte. It was observed that for LC-MS/MS analysis, recovery of polar polyoxin D was affected by the matrix interference in terms of signal suppression which resulted in quantification errors, as grape matrix consists of high content of polyphenolic compounds, sugars and pigments (Mirabelli et al., 2018). The Matrix effects (ME) are an important drawback of LC-MS/MS analysis, due to the presence of co-eluting components (Chamkasem and Hormon, 2016; Chen et al., 2013; Nortes-Mendez et al., 2016; Pizzutti et al., 2016). To minimize these errors and obtain satisfactory results, the matrix effect (ME) was calculated by comparing the slope of the calibration curve based on the matrix-fortified standard with the calibration curve of the matrix-free pure solvent-based standard. This validated method was thus successfully employed for the analysis of field samples collected from four different agro climatic locations of India for polyoxin D in grape.

3.2.2. Method validation

The selectivity of the developed method was affirmed by the identical retention times and mass spectra of the standard (in solvent) and blank sample fortified with standards. For accurate quantitation, matrix-matched calibration was prepared, which provided excellent linearity with a coefficient of determination (R^2) ≥0.999. The linearity of the calibration curve was evaluated in for the 0.001, 0.05 and 0.1 mg kg^{-1} for polyoxin D with a correlation coefficient (R^2) of the calibration curve of >0.99. The LOQs of polyoxin D was 0.01 mg kg^{-1}. The average percentage recoveries of polyoxin D at 0.01, 0.05, and

0.1 mg kg^{-1} were (83.76±6), (85.45±5), (86.35±5) for grape. The matrix effect was reflected in terms of signal suppression by ≥23% and therefore, matrix matched calibration was used for its precise quantification.

3.2.3. Dissipation

The polyoxin D analysis method was developed in grape berries and applied for the analysis of grape samples collected from field trials. The dissipation behavior of polyoxin D pertaining to recommended and double recommended dose treatments are presented in Figure 1. Dissipation data of polyoxin D pertaining to the recommended and double recommended dose, i.e.,

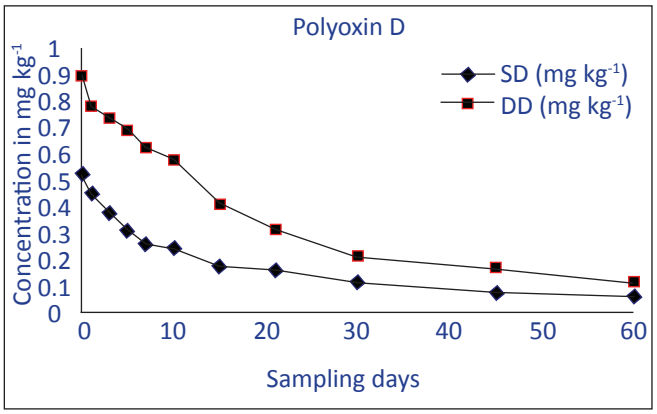

Figure 1: Dissipation of polyoxin D in grape (SD: Recommended dose DD: Double dose)

30 and 60 g a.i. ha^{-1}, in grape, are presented in Table 4 and representative chromatograms are provided in Figure 2. The initial residue deposits were 0.52 and 0.89 mg kg^{-1} at single and double doses, respectively. A gradual and continuous degradation of the residues was observed with time as expected. The day-wise residue data had excellent fit to 1st+1st order models giving r^2 value of > 0.99 as compared to 1st order model. The residue dissipation data given in Table 3 suggested that the half-life (t $_{1/2}$) period of polyoxin D in grape samples were 8.0 days for recommended dose and 14.5 days for double dose, clearly indicating its low duration of retention. In India, food safety is based on the guiding principle of risk analysis of the Codex Alimentarius Commission (CAC). In order to exploit full potential of pesticides in agriculture and public health programmes without adversely affecting the environment, it is mandatory to study the facts about pesticide behavior and their dissipation under tropical Indian conditions, along with consumer safety and overcoming of trade barriers at international level (Anonymous, 2018). It is worth mentioning that the MRL of polyoxin D in agricultural products are wanting in national and international databases (Anonymous, 2016; Anonymous, 2009; Anonymous, 2015). For registration of polyoxin D, such a field study was carried out to evaluate the safety and efficacy of compound (Anonymous, 1986) which in turn will assist in establishing MRL for grape in India.

Table 4: Degradation kinetics of polyoxin D in grape

Days after spray		Grapes	
		SD	DD
0		0.522	0.894
1		0.452	0.774
3		0.378	0.737
5		0.312	0.692
7		0.260	0.627
10		0.244	0.581
15		0.175	0.408
21		0.160	0.314
30		0.115	0.211
45		0.074	0.169
60		0.061	0.115
1st order	[A]1 (mg kg^{-1})	0.4675	0.848
	k1 (Day^{-1})	0.0595	0.0431
	[A]2 (mg kg^{-1})	NA	NA
	k2 (Day^{-1})	NA	NA
	R^2	0.9428	0.9834
	DT$_{50}$ (Day)	11.5	16
1 st+ 1 st order	[A]1 (mg kg^{-1})	0.2542	0.7809
	k1 (Day^{-1})	0.2285	0.0551
	[A]2 (mg kg^{-1})	0.2640	0.0852
	k2 (Day^{-1})	0.0265	0.1e-12
	R^2	0.9968	0.9899
	DT$_{50}$ (Day)	8	14.5

SD: Single dose; DD: Double dose; r^2- Correlation coefficient; DT$_{50}$: Time for 50% degradation

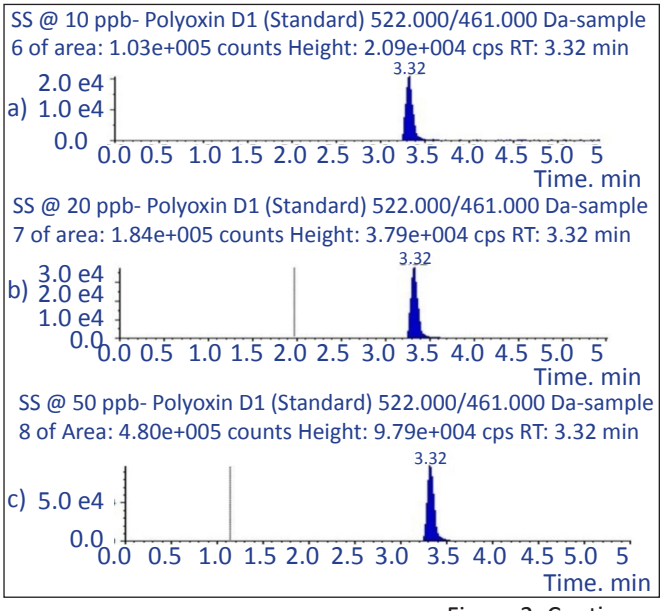

Figure 2: Continue..

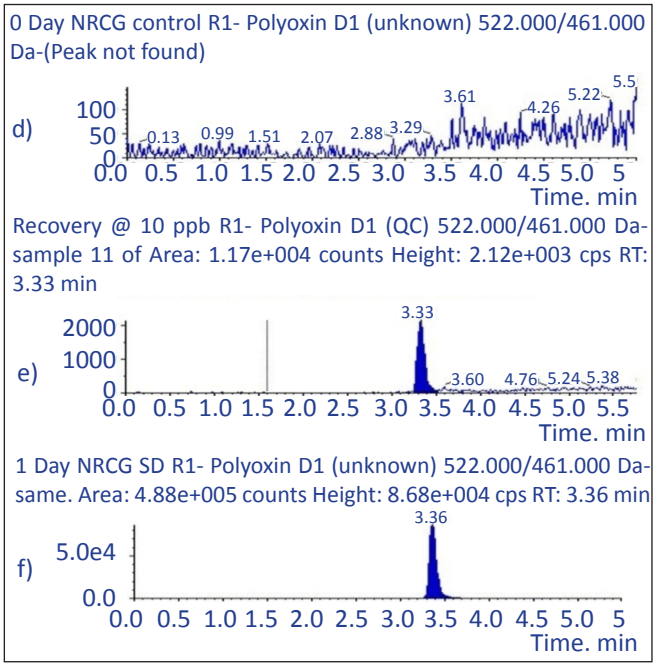

Figure 2: LC-MS/MS chromatograms of polyoxin D: (a) standard spiked at 0.01 mg kg^{-1} b) standard spiked at 0.02 mg kg^{-1} and c) standard spiked at 0.05 mg kg^{-1}. d) Grape control 0 day (e) fortified sample at 0.01 mg kg^{-1} and (f) single-dosed field-treated sample of 1 day).

4. Conclusion

The fungicide would be the safe that could reduce disease incidence @ 600 ml ha^{-1} than Flusilazole 40 EC, Hexaconazole 5 EC and Myclobutanil 10 WP. The analytical method is reliable. The dissipation dynamics revealed that the half-life period of polyoxin D in grape samples were 8.0 days for recommended dose, indicating its low duration of retention. The method can be easily practiced for routine analysis of polyoxin D in grape. This will contribute to establish the MRLs of polyoxin D for grape in India.

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