PROJECT REPORT

EVALUATION OF THE RESIDUE AND PERSISTENCE STUDY OF ProPhite (PSAP) IN/ON GRAPE

Sponsored By

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2019-20

Test Facility

National Referral Laboratory

ICAR-NATIONAL RESEARCH CENTRE FOR GRAPES
(Indian Council of Agricultural Research)
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Study Title

EVALUATION OF THE RESIDUE AND PERSISTENCE STUDY OF ProPhite (PSAP) IN / ON GRAPE

Study Purpose

Test Item	**ProPhite (PSAP –Potassium Salt of Active Phosphorous)**
Test System	Grape berries and soil
Test Facility	ICAR-National Research Centre for Grapes, Pune
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SUMMARY

This study determined residues of ProPhite (PSAP) as Phosphonic acid in/on grape berries and soil in India following treatment with ProPhite (PSAP) as foliar spray. Field trials were carried out during the 2019-2020 crop seasons at the A/P-Malegaon, Baramati under the ICAR-NRC for Grapes, Pune.

ProPhite (PSAP) was applied by foliar application method at the time of berry formation @ 4 g/L (T1) and 8 g/L (T2). Total three sprays were at 7 days interval. An untreated control plot (T0) was maintained for comparison. Field experiment plots were laid in Randomized Block Design (RBD) with three replications for each treatment. Samples of grape berries were collected at 0 (within 2 hours), 1, 3, 5, 7, 10, 15, 21, 30, 45 and 60 day(s) (Harvest) after final application. Soil was sampled at 60 days after the final applications.

Residue analysis of ProPhite as Phosphonic acid was carried out at the NRL of ICAR-NRCG, Pune using LC-MS/MS method, which provided a limit of determination/quantification of 0.01 mg/kg for phosphonic acid in grape berries and soil. The limit of detection (LOD) of phosphonic acid was 0.003 mg/kg for grape berries as well as soil.

The recovery experiment was carried out on the control samples by fortifying the fresh untreated grape berries and field soil matrix with 0.01, 0.05 and 0.10 mg/kg of analytical standard of phosphonic acid solution. The spiked samples were extracted and analyzed by the method described below. The per cent recovery was calculated by comparing with standards using an eight point calibration curve. The data is presented in the Table 1 for both grape berries and soil. The recoveries of phosphonic acid during method validation was acceptable with relative standard deviations of <10%.

Table 1: Recovery for phosphonic acid in grape berries and cropped Soil

Grape		Soil	
Level of fortification (mg/kg)	**Recovery (%)**	**Level of fortification (mg/kg)**	**Recovery (%)**
0.01	79.67 ± 0.94	0.01	78.77 ± 1.64
0.05	82.94 ± 1.88	0.05	80.41 ± 1.98
0.10	85.80 ± 1.92	0.10	83.80 ± 1.77

Residue data obtained from field experiment of ProPhite (PSAP) is summarized in **Table 2.**

Table 2: Residue study of phosphonic in grape berries and soil

Phosphonic acid residues (mg/kg)			
Grapes Berries			
Sampling (Days)	**T0**	**T1**	**T2**
0	<LOQ	14.50	28.93
1	<LOQ	12.33	23.50
3	<LOQ	9.85	22.20
5	<LOQ	8.89	21.06
7	<LOQ	8.36	20.10
10	<LOQ	8.07	16.03
15	<LOQ	6.70	14.43
21	<LOQ	4.73	10.36
30	<LOQ	1.58	4.04
45	<LOQ	1.01	2.82
60 (Harvest)	<LOQ	0.81	2.25
Correlation Coefficient (r^2)	-	0.98	0.98
Half-life (days)	-	10.5	14.5
Grapes soil			
60 (Harvest)	<LOQ	<LOQ	<LOQ

Limit of quantification (LOQ) ≤ 0.01 mg/kg

FIELD EXPERIMENTAL PROCEDURES

The residue field trial was conducted at ICAR-National Research Centre for Grapes, Pune.

The field experimental plots were laid in Randomized Block Design (RBD) with three replications for each treatment. The formulation of ProPhite (PSAP) was applied on grape vines as foliar spray at;

T0- An untreated control for comparison

T1- ProPhite (PSAP– Potassium Salt of Active Phosphorous) @ 4000 g/ha formulation (X)

T2- ProPhite (PSAP– Potassium Salt of Active Phosphorous) @ 8000 g/ha formulation (2X)

The grape berry samples were collected at 0 (within 2 hours), 1, 3, 5, 7, 10, 15, 21, 30, 45 and 60 (harvest) days following the last applications. Soil was sampled on 60th day after the final application. Residue analysis of ProPhite was carried out at ICAR-NRCG using a validated LC-MS/MS method.

The Field experiment details are as follows:

LOCATION - ICAR-National Research Centre for Grapes, Pune.

GENERAL INFORMATION	
Test item	ProPhite (PSAP – Potassium Salt of Active Phosphorous)
Nature of chemical	Stress Alleviator and Activator
Type of formulation	00 : 40% : 40% (N:P:K)
Type of trial	Field residue study
Commodity	Grape berries & Cropped soil
Variety	Table grapes, Variety-Thompson seedless
APPLICATION DATA	
Crop	Grape (*Vitis vinifera L*)
Date of Pruning	02.12.2020
Description of the plot plan	All the experimental plots were laid in Randomized Block Design
Number of vines/ plot	10
No of plots/ treatment	3
Number of rows/ plot	-
Spacing (row to row × vine to vine)	3 m × 1.5 m
Number of control plots	-
Growth stage of application	Berry formation stage
Method of application & equipment	Foliar application with Knapsack pump using hollow cone nozzle
APPLICATION DETAILS	
No. of applications	Three (3) at 07 days interval
Date of applications	1st Spray- 03.02.2020 2nd Spray- 10.02.2020 3rd Spray- 17.02.2020
Application rate	T0- Untreated Control T1- ProPhite (PSAP – Potassium Salt of Active Phosphorous @ 4000 g/ha T2- ProPhite (PSAP – Potassium Salt of Active Phosphorous @ 8000 g/ha
Water volume	1000 L/Hectare
SAMPLING DATA	
No. of samples taken per treatment	Three
Method of sampling	Grape Berries: The berry samples were randomly collected from 20 bunches or parts of bunches from at least 4 different vines to give at least 1 kg of material per treatment.

		Soil: Soil core samples (15 cm depth) were taken from a minimum of 12 spots of the plot, composited, thoroughly mixed and then sub-sampled to obtain 500 g sample.
Dates of Sampling	**Days after the final application**	**Date of Sampling**
	0	17.02.2020
	1	18.02.2020
	3	20.02.2020
	5	22.02.2020
	7	24.02.2020
	10	27.02.2020
	15	03.03.2020
	21	08.03.2020
	30	18.03.2020
	45	02.04.2020
	60	17.04.2020
Date of soil sampling	60	17.04.2020
Storage Conditions before analysis		-20 ± 1°C

ANALYTICAL METHOD

Materials

Apparatus

1. Instrument method details: Shimadzu UFLC coupled to API 5500 Qtrap MS/MS (Sciex) System, controlled by Analyst® 1.7.1 software
2. Column: Phenomenex, Luna, 150 × 4.6 mm, 5.0 µm particle Size
3. Electronic balance (Vibra, Adair Dutt, Mumbai, India)
4. Precision electronic balance (Vibra, Adair Dutt, Mumbai, India)
5. Vortex Scientific Industries (Geni2T, Imperials Biomedicals, Mumbai, India)
6. Centrifuge- High Volume (Kubota, Germany)
7. Centrifuge- Low Volume (Microfuge Pico, Kendro, D-37520, Osterode, Germany)
8. Ultrasonic Bath (Oscar electronics, Mumbai, India)

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GLASSWARE & EQUIPMENT CLEANING PROCEDURE

All the glass items were initially rinsed with acetone and then cleaned with an aqueous soap solution. Then, the glassware was rinsed in tap water followed by acetone rinse and dried in a hot air oven.

REAGENTS AND CHEMICALS:

Water (HPLC grade), Formic Acid (Sigma Aldrich), Methanol (LC-grade from J.T. Baker), Ammonium formate (Thomas Baker) were used at different stages of sample preparation.

TEST PROCEDURES

Preparation of calibration and fortification standards:

Quantification of the residues was done by an external calibration method. The stock solution, 1000 mg/L, was prepared by dissolving 0.1 g of phosphonic acid standard in 100 mL water. An intermediate standard solution of 10 mg/L was prepared by diluting 1 mL of the stock solution with methanol in a 100 mL volumetric flask. From this intermediate standard solution, a set of eight-point calibration standards were prepared by dilution in solvent and in blank matrix. The standard curve was obtained by plotting the peak areas against the concentration levels of the calibration standards.

SAMPLE PREPARATION

The entire sample was crushed in a homogenizer thoroughly.

Extraction procedure for grape sample

The homogenized grape sample (10 ± 0.1 g) was taken in a 50 mL centrifuge tube and to it 20 mL methanol consisting 1 % formic acid was added. The mixture was homogenized for 1 min and centrifuged for 5 min at 10000 rpm. The clear supernatant was diluted (1:1) with methanol: water, filtered through a 0.2 µm nylon membrane filter, and then injected into the LC-MS/MS system.

Extraction procedure for soil sample

The soil sample (5 ± 0.10 g) was taken in a 50 mL centrifuge tube and to it 20 mL methanol consisting 1 % formic acid was added. The mixture was shaken vigorously for 1 min and centrifuged for 5 min at 10000 rpm. The clear supernatant was diluted (1:1) with methanol: water, filtered through a 0.2 µm nylon membrane filter and then injected into the LC-MS/MS system.

The samples showing residues above the highest calibration standard were diluted and injected to fit into the calibration linearity. Appropriate dilution factor was applied while quantifying the residues in such samples.

LC-MS/MS:

Shimadzu UFLC coupled to API 5500 Qtrap MS/MS (Sciex) System, controlled by Analyst® 1.7.1 software.

Column: Phenomenex, Luna, 150×4.6 mm, 5.0 µm particle Size

Injection Volume- 10 µL

Retention time- 7.10 minutes

Calculations:

The analyte concentrations were calculated using the Analyst® 1.7.1 software. The software calculates the standard curve and applies the dilution factor to account for sample weight and dilution volume. Linear calibration curves were used for quantitation.

Linear Calibration Curve: The Analyst® software automatically derives the calibration curve using the area response (y) versus the concentration (x) of the external standards for all standards injected with the chromatographic set. A weighted linear regression (1/x) standard curve was used. The resulting equation defining the standard curve is shown below:

$y = Ax + B$ where,

x = concentration injected (ug/mL)

y = detector response (peak area)

A = slope
B = Intercept

RESULTS & DISCUSSION:

LINEARITY

The calibration curves were found to be linear with correlation coefficients greater than 0.999 when tested in the range of 0.001 to 0.100 mg/kg for phosphonic acid.

FORTIFICATION RECOVERIES

Grape and soil

The method for phosphonic acid was validated for grape and soil samples fortified at 0.01 mg/kg, 0.05 mg/kg and 0.10 mg/kg. Recovery data from the method validation were acceptable, i.e. the average recoveries at each fortification level were within 79.67-85.80% for grape and 78.77-83.80% for soil with relative standard deviations of <10% for phosphonic acid in **Table 1a.**

RESIDUES:

The residues of ProPhite in grape berries and soil at different sampling intervals are presented in **Table 2a.** Analysis of grape samples collected on day 0 showed 14.5 mg/kg and 28.93 mg/kg of phosphonic acid residue in T1 (x dose) and T2 (2x dose) applications, respectively. The residue levels on 15th day were at 8.07 mg/kg and 16.03 mg/kg in T1 (x dose) and T2 (2x dose) applications, respectively. On the 30th day, the residues had dissipated to 1.58 and 4.04 mg/kg. By 60th (harvest) day, the residues had further dissipated to 0.81 mg/kg and 2.25 mg/kg in T1 (x dose) and T2 (2x dose) applications, respectively. The ProPhite residues were below the limit of quantification in soil at both dosages of T1 and T2.

Half-life:

The dissipation behavior of ProPhite in grape samples was faster in the beginning, which slowed down with the passage of time. This indicated a non-linear pattern of degradation and

implies that the simple 1st order kinetics might not be adequate to explain the dissipation behavior of ProPhite residues in grape. Hence, the kinetics of residue data was evaluated by fitting the data into non-linear 1st + 1st order model for estimation of the half-life. The half-life values were 10.5 and 14.5 days for T1 and T2 dose respectively.

CONCLUSION

The dissipation behavior of ProPhite in grape berries shows that the residues declined rapidly, in the initial 1st phase, which slowed down with the passage of time in the later phase. The ProPhite (Phosphonic acid) residue dissipated with a half-life of 10.5 and 14.5 days for T1 and for T2 dose, respectively. No residues of ProPhite (Phosphonic acid) were detected in soil collected from T1 and T2 dosages.

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ANNEXURE I

Table 1a: Recovery data of ProPhite (Phosphonic acid) in grape and soil

Recovery (%) in grape berry					
Level of fortification (mg/kg)	**R1**	**R2**	**R3**	**Average**	**% RSD**
0.01	80.39	79.66	78.92	79.67	0.94
0.05	81.18	84.30	82.99	82.94	1.88
0.10	87.50	84.90	85.01	85.80	1.92
Recovery (%) in soil					
Level of fortification (mg/kg)	**R1**	**R2**	**R3**	**Average**	**% RSD**
0.01	78.08	80.30	77.94	78.77	1.64
0.05	82.17	79.00	80.29	80.41	1.98
0.10	83.90	84.90	82.01	83.80	1.77

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ANNEXURE II

Table 2a. Degradation of the residues of ProPhite (Phosphonic acid) in grapes berries and soil

Residue content (mg/kg)															
Days	**T0R1**	**T0R2**	**T0R3**	**Mean**	**SD**	**T1R1**	**T1R2**	**T1R3**	**Mean**	**SD**	**T2R1**	**T2R2**	**T2R3**	**Mean**	**SD**
Grape berries															
0	BLQ	BLQ	BLQ	BLQ	-	13.00	14.90	15.60	14.50	1.35	27.80	28.90	30.10	28.93	1.15
1	BLQ	BLQ	BLQ	BLQ	-	12.10	12.70	12.20	12.33	0.32	23.10	23.10	24.30	23.50	0.69
3	BLQ	BLQ	BLQ	BLQ	-	9.57	9.60	10.40	9.86	0.47	20.30	23.00	23.30	22.20	1.65
5	BLQ	BLQ	BLQ	BLQ	-	9.14	8.90	8.63	8.89	0.26	21.30	20.80	21.10	21.07	0.25
7	BLQ	BLQ	BLQ	BLQ	-	8.23	8.53	8.34	8.37	0.15	20.20	20.00	20.10	20.10	0.10
10	BLQ	BLQ	BLQ	BLQ	-	7.86	8.32	8.04	8.07	0.23	15.60	16.60	15.90	16.03	0.51
15	BLQ	BLQ	BLQ	BLQ	-	6.53	7.00	6.58	6.70	0.26	14.70	14.40	14.20	14.43	0.25
21	BLQ	BLQ	BLQ	BLQ	-	4.38	4.95	4.86	4.73	0.31	10.20	10.60	10.30	10.37	0.21
30	BLQ	BLQ	BLQ	BLQ	-	1.56	1.59	1.58	1.58	0.02	4.02	4.03	4.07	4.04	0.03
45	BLQ	BLQ	BLQ	BLQ	-	0.97	1.02	1.03	1.01	0.03	2.70	2.86	2.90	2.82	0.11
60 (Harvest)	BLQ	BLQ	BLQ	BLQ	-	0.76	0.85	0.79	0.81	0.05	2.01	2.30	2.44	2.25	0.22
Soil															
60 (Harvest)	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ

SD: Standard Deviation

BLQ: Below limit of quantification ≤ 0.01 mg/kg